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WINTER HEATING EFFECTS ON PLANTS PERFORMANCES, GROWTH AND PHENOLOGY

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Abstract

Climate change is unprecedently threating living organisms. The increasing of carbon dioxide in the atmosphere is the major driver of climate change, causing a dramatic rise of temperature. Global warming and high CO_2 concentration have significant consequences on plants performances. Most of the studies focus on the effect of climate change on growing season, since photosynthesis during winter is negligible. However, mild winters are becoming more frequent and plants performances could become more noteworthy. At high-latitudes where milder winters are linked to wider cloud cover plants respiration could exceed photosynthesis causing a negative carbon balance. Nonetheless, temperature rise during winter could affect growth and phenology leading to less carbon storage and phenological mismatches. To understand plants performances, growth and phenology under mild winter five species of seedlings (*Picea abies*, *Abies alba*, *Larix X eurolepis*, *Fagus sylvatica* and *Quercus robur*) were set under different temperature and light treatments indoor and outdoor for one month between January and February. Plants performances were measured on evergreen conifers during the whole month. An increased level of dark respiration and a lower carbon uptake was found in plants exposed to warmer temperatures. Growth and phenology were monitored on the five species revealing species-specific responses. An overall advancement in phenology was observed in plants placed at warmer temperatures. Light treatments triggered a phenology advancement in *Picea abies* and *Quercus robur*. This study evidences the importance of including winter temperatures and light to calculate annual carbon balance, and plants growth and phenology.

Keywords: winter photosynthesis • global warming • conifer respiration • carbon uptake • phenological advancement

Resumo

As alterações climáticas são uma das principais ameaças à sobrevivência de várias espécies. Estima-se que a temperatura média da superfície da Terra aumente 1.5ºC entre os anos de 2030-2050. Prevê-se que esta temperatura aumente especialmente em latitudes mais altas, atingindo mais 4.5ºC. Além disso, o aumento do dióxido de carbono atmosférico está a alterar os ciclos de carbono, levando a consequências que são altamente complexas de assimilar. As florestas representam um papel fundamental para os ciclos gasosos, sendo um dos maiores sumidouros de carbono do planeta. Como o desempenho das plantas pode ser afetado por elevadas temperaturas e por elevadas concentrações de CO2, há incertezas sobre a conservação das florestas como sumidouros de carbono, sem que as mesmas se tornem fontes de carbono. Assim sendo, pode eventualmente verificar-se um efeito reverso no papel das florestas sob resultado do aquecimento global.

A fotossíntese é o único processo que converte energia solar, dióxido de carbono e água em carboidratos não estruturais e oxigénio molecular. Desta forma, a energia solar é convertida em energia química, que pode ser utilizada por organismos heterotróficos como fonte primária de alimento. A fotossíntese é fortemente influenciada pelo ambiente, uma vez que esta responde a fatores ambientais como luz, concentrações de CO_2 e temperatura. Entre eles, o efeito da temperatura é particularmente interessante porque envolve alterações em todas as etapas da fotossíntese.

A temperatura é um fator capaz de alterar as atividades das enzimas da cadeia da fotossíntese, criando respostas amplas e diferentes. No entanto, não é apenas a temperatura que leva a impactos consideráveis ao nível da fotossíntese. A luz é a responsável por fornecer a energia necessária para o processo fotossintético, desempenhando por isso um papel altamente importante no desempenho das plantas. O espectro de luz que pode ser usado pelas plantas para fazer a fotossíntese é chamado de radiação fotossinteticamente ativa (PAR).

No contexto do presente estudo, as temperaturas mais elevadas estão principalmente associadas a clima nublado, o que modifica a PAR. Esta diminui com condições de nebulosidade considerável, o que corresponde a uma consequente diminuição da taxa de transpiração e aumento da taxa de fixação de dióxido de carbono.

Temperatura e radiação luminosa são dois fatores altamente importantes a serem observados no cenário de mudanças climáticas. Como consequência, a combinação destes dois fatores mostra respostas complexas nas plantas, podendo não só afetar o desempenho das mesmas no inverno, mas também influenciar o processo normal do seu crescimento e da sua fenologia. A monitorização destes dois parâmetros e o estudo dos seus efeitos no desempenho da fotossíntese e, consequentemente, no desenvolvimento das plantas, é crucial para entender os verdadeiros efeitos das alterações climáticas a nível global.

O presente estudo pretende explorar esta temática, de forma a contribuir para a compreensão dos efeitos do aquecimento global, no inverno, em cinco espécies de árvores que estão amplamente distribuídas pela Europa: *Picea abies, Abies alba*, *Larix X eurolepis, Fagus sylvatica e Quercus robur*. As experiências decorreram em Horshlom, Dinamarca.

Dois processos experimentais foram montados, de forma a expor as respetivas plantas a um ambiente com temperaturas acima do normal, durante o período de 1 mês. Por um lado, pretendia-se testar a

resposta das plantas num ambiente natural com um ligeiro, mas significativo, aumento de temperatura, por outro, pretendia-se tornar essas condições extremas, através de criação de um ambiente controlado e manipulável. Assim, 320 plantas foram selecionadas aleatoriamente para uma experiência ao ar livre e 168 para uma experiência em ambiente fechado.

Ao ar livre foram preparadas 8 parcelas de 12 m2, onde metade delas foi aquecida com 6 aquecedores e a outra metade funcionou como controlo. Metade de cada parcela foi ainda coberta com uma rede para reter 60% da luz e criar um ambiente de sombra. Pretendia-se manter as parcelas aquecidas a 4ºC em comparação com o controlo, utilizando um computador capaz de manter a diferença constante ao longo do tempo.

Na experiência em ambiente fechado, colocaram-se as plantas em quatro estufas diferentes e uma parcela de controlo externa. As estufas foram aquecidas a uma temperatura média de 13°C - 11°C - 9°C - 6°C. Em cada estufa foram simulados ambientes diferentes: metade das plantas recebeu um nível de luz ambiente e a outra metade foi isolada de fatores luminosos.

A experiência ocorreu de 7 de janeiro a 7 de fevereiro. Durante o mês experimental, foram realizadas medições de trocas gasosas com CIRAS-3 em coníferas perenes (*Picea abies e Abies alba*), ou seja, foram realizadas curvas de temperatura e luz, medição de luz ambiente e curvas diurnas todas as semanas durante quatro semanas.Após o mês experimental, as plantas foram movidas para um terreno ao ar livre e outro interno, numa estufa mais fria (respetivamente para as plantas pertencentes ao experimento ao ar livre e ao experimento interior). Estas foram colocadas aleatoriamente para o começo da estação de crescimento. Durante a primavera, efetuaram-se medições de crescimento e de fenologia. Para a avaliação do crescimento, a altura e o diâmetro foram medidos antes e depois da estação de crescimento, enquanto que a fenologia foi medida através de métodos de pontuação durante a primavera.

Numa primeira análise, foi possível avaliar que a diferença de temperatura entre as parcelas aquecidas e as de controlo, na experiência ao ar livre, foi de apenas 1,9°C. Foi também possível demonstrar que os resultados foram menos significativos ao ar livre do que na experiência em estufas, onde as temperaturas estabelecidas conseguiam ser facilmente alcançadas.

A análise estatística dos dados de trocas gasosas revelou um forte efeito da temperatura no desempenho das plantas. As curvas de temperatura e de luz evidenciaram que as plantas implantadas em ambientes mais frios apresentaram melhor desempenho do que as demais, e os níveis de respiração em zonas de luminosidade reduzida aumentou em ambos os tratamentos de temperatura. Dentro dos modelos criados para as respirações com luminosidade reduzida, verificou-se que, especialmente na experiência interna, a respiração estava a aumentar exponencialmente com o efeito do aumento de temperatura. Além disso, verificou-se também que a espécie *Picea abies* teve uma taxa respiratória mais alta do que a espécie *Abies alba*.

A absorção de carbono também foi afetada pela temperatura. Foi possível notar uma diminuição exponencial da fixação de carbono com o aumento da temperatura na experiência interna. Por sua vez, as plantas colocadas em temperaturas mais elevadas durante o inverno tiveram uma menor absorção de carbono. Além disso, as plantas que cresceram em temperaturas mais elevadas mostraram uma maior diversidade e complexidade de respostas, o que significa que a variabilidade parece aumentar com a temperatura. Por este motivo, prever a precisão das respostas das plantas a temperaturas mais altas irá tornar-se cada vez mais complexo e incerto a longo prazo.

Finalmente, foi efetuada uma análise do efeito dos tratamentos de temperatura e luz no crescimento e fenologia das plantas. Os resultados mostraram que as respostas são altamente específicas e intrínsecas de cada espécie. Algumas das espécies conseguiram beneficiar de temperaturas mais altas enquanto que outras se mostraram mais afetadas. O *Larix X eurolepis* foi afetado negativamente pela temperatura, ao contrário do *Fagus sylvatica*, que aumentou o seu crescimento quando exposto a tratamentos mais quentes. A luz afetou diferencialmente o *Quercus robur*, onde este cresceu mais quando exposto à luz ambiente dentro das estufas, e menos ao ar livre. Tudo isto veio suportar a ideia de que as respostas das plantas podem variar significativamente dependendo das diferentes condições a que são submetidas.

Foi possível adequar modelos fenológicos apenas para a o ensaio interno. Infelizmente, a generalização de dados do ensaio ao ar livre não permitiu uma análise precisa, por isso não foi possível avaliar a fenologia dessas plantas. Além disso, as plantas instaladas em estufas evidenciaram um avanço em eventos de primavera, quando expostas a temperaturas mais elevadas no inverno. Em especial, *Quercus robur* e *Picea abies* tiveram cerca de 14 dias de avanço entre o tratamento mais quente e o mais frio. O tratamento com luz teve um efeito sobre *Quercus robur* e *Picea abies*, onde ambos responderam negativamente à ausência de luz durante o mês experimental com um atraso geral dos eventos de primavera.

Para concluir, foram observados efeitos da temperatura no desempenho das plantas durante o inverno e no crescimento e fenologia durante a primavera. Assim sendo, os dados correspondentes às performances de plantas em invernos amenos devem ser incluídos nos estudos de modelos do balanço anual de carbono e, as incompatibilidades de crescimento e fenologia deverão ser esperadas em cenários de aquecimento global. As entidades responsáveis pela tomada de decisões a nível mundial devem levar em consideração tais resultados, de forma a melhorar as práticas de gestão florestal e tentar combater assim os efeitos adversos das alterações climáticas.

Palavras-chave: fotossíntese de inverno • aquecimento global • respiração de coníferas • absorção de carbono • avanço fenológico

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Motivation

It was the beginning of March 2018, my last day of internship in Magoodhoo, Maldives. I was swimming with my closest buddies observing the seabed in the shallow waters of Indian ocean during the sunrise. The atmosphere was just incredible. The night before, we had the chance to see an amazing documentary – Chasing Corals – made by Jeff Orlowski. The documentary told about the heatwaves of 2015-2016 that killed a big portion of coral reefs all around the world. The images were so dramatic and powerful that none of us was able to look at the Earth and nature in the same way that we did before. That morning, merged in the silent waters, I was seeing the wideness of climate change consequences. Corals were all bleached. I felt the indescribable damage that human beings have been doing to all the other living organisms. Coral reefs, amazing structures, symbol of hundreds of thousands of years of carbonate deposition were all dead. Only few corals had the strength to survive. Fishes and other sea organisms were wandering around desperate for seeking food. There were no words to describe the feeling of losing such a beauty and a sink of biodiversity because of our arrogance. I hadn't realised the intensity of the damage before that documentary, I thought that the grey looking of the reefs was just a normal thing, that all the colours that I always dreamed about were an exaggeration of the media. But that was not the case. That grey was the symbol of the power of humankind to disrupt ecosystems. My tutors were doing their Ph.D. on coral reefs in Maldives during the big heatwave of 2015-2016. They could tell what they had been seeing disappear under their eyes. You could see the feeling of powerlessness in their stricken looks. But they stood up for this cause and they started to work harder to find strategies to fix what was happening. It looks like a war against an invisible but huge enemy, a fight against time and forces that are so difficult to control.

It was there that I felt the need to embrace this cause, to protect nature, to study more and more to understand the biology, the policies and the economics behind ecosystems wellness. I wanted to learn everything about climate change and what strategies and innovative solutions we could find to protect such a fragile and fascinating thing: life. Since then, I decided to take a Master in Ecology and Environmental Management at the University of Lisbon that gave me the chance to learn the multifaced consequences of climate change and the action plans that can be achieved through political decisions making. All these choices brought me to the faculty of Geoscience and Natural Resource Management at the University of Copenhagen to study the effect of climate change on plants physiology with an ecophysiological point of view. A research project that was looking into the effects of climate change at all the levels. I was always fascinated by botanic, plants evolution and physiology, and to be able to work to understand the effect of global warming on these key living organisms was a true realization. I know that all the steps that brought me here are just the beginning. I want to explore more and to take action in such a fragile time, where life as we know it, is deeply threatened.

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1. Introduction

Among infinite shapes of living organisms on Earth, major plants cover a key role in the ecosystems. Transforming solar energy into chemical through the photosynthesis, they are the first energy input for all the other heterotrophic organisms. Therefore, they not only become an irreplaceable source of food, but through this major biochemical process they produce molecular oxygen that allows the existence of aerobic forms of life. For this reason, the interaction of different parts in this game is essential to understand life on Earth. They, in fact, create the ecological complexity that is crucial for biodiversity. Climate change is unprecedentedly threatening the subtle equilibrium of life that evolved so far, at different levels. One of the most interesting and complex consequence of climate change is the increasing of temperature. Even though, a couple of degrees more look imperceptible to humankind, they are able to disrupt ecosystems. The understanding of the multiple and multifaceted responses of plants to this issue is crucial for the survival of many species, including *Homo sapiens*. This project wants to embrace this challenge and to question the consequences of temperature increasing on plants with an ecophysiological approach.

1.1 Climate change and forest ecophysiology

Global mean surface temperature (GMST) has increased of 0.87°C in the period between 2006-2015, compared to 1850-1900 (Hoegh-Guldberg *et al.*, 2018). If the temperature will continue to rise in the current way, it is expected to reach 1.5°C more between 2030-2052 (IPCC, 2018). Consequences of temperature rising could be very severe, there are already visible damages on organisms, ecosystems and human systems, and well-being (Hoegh-Guldberg *et al.*, 2018). The frequency and magnitude of events able to irreparably create strong impacts are increasing. They could reach the tipping point where there is no turning back. Projections that are more optimistic already show dramatic scenarios. Global warming of 1.5°C refers, indeed, to an average increasing of mean temperature both on land and in oceans. Oceans are likely to rise temperature slowly because of the chemical properties of water. Despite, temperature is rising above this threshold on land, due to the land-see contrast in warming. This implies that it is fundamental to analyse global warming at a more regional scale (Hoegh-Guldberg *et al.*, 2018). Global scale projections, indeed, tend to underestimate regional changes (Seneviratne *et al.*, 2016), meaning that some of the impacts will be stronger depending on the subjected area. Nevertheless, general assessments reveal that number of cold days and nights will diminish, accompanied by an increase of warm days and nights at a global scale (Hoegh-Guldberg *et al.*, 2018). But considering that consequences could be more or less strong depending on the area, it is important to understand where the impacts will be greater. One of the areas that is likely to suffer the strongest warming of mean temperatures and cold extremes is at northern latitudes (IPCC, 2013), where the expected warming is above the global average. Projections estimate that it will rise up to 4.5°C during the cold season (Hoegh-Guldberg *et al.*, 2018).

Some of the most dramatic consequences of a higher GMST regard land degradation, with a continue increasing of vegetation loss (Hurlbert *et al.*, 2019). Temperature rise affects physical and biological systems. Therefore, distribution, abundance, migration and patterns of animals and plants species will be strongly changed. Some of the consequences concern an earlier spring or a shift in cool or warm adapted species (WIREs, 2013). Since the temperature will rise with different intensities, some of the living organisms could not be able to adapt, and some others will adapt better at higher latitudes. Many studies reported, indeed, a latitudinal and elevational shift of biomes (Settele *et al.*, 2014). It is expected that 6.5% of biomes could be transformed with 1.5°C temperature

rise (Hoegh-Guldberg *et al*., 2018). Ecosystems dynamics and responses to climate change are very complex to predict. Ecosystems, in fact, have undefined and variable boundaries, different species composition, and a continue flow of energy, organisms and materials among each other. They are extremely diversified and interconnected. Human activities must be considered as an integrated part of the ecosystems. Hence, they can significantly impact their functionality (Settele *et al*., 2014). Understanding major drivers and effects of climate change in the ecosystems at local and global scale has a key role to improve management practices and to reduce climate change impacts (WIREs, 2013).

With regard to terrestrial ecosystems under global warming, there are three main aspects that worth to be analysed: shifts in phenology, changes in species range abundance and extinction, and variation in ecosystems function, biomass and carbon stocks (Hoegh-Guldberg *et al*., 2018). In the report *Climate change 2014: Impacts, Adaptation and Vulnerability* (Settele *et al*., 2014) has been evidenced a 'Spring advancement' especially in Northern Hemisphere. An overall estimation shows that phenological spring events are advancing -2.8 ± 0.5 days every decade. Among the others, trees show the major advancement, meaning -3.3 ± 0.87 days every decade (Parmesan, 2007). Considering this scenario there is a potential to have a phenological mismatch among species. Namely, there is a very tight link between animals and plants phenology. E.g., if an advancement of blooming is not followed by an advancement of bee's development, there could be a huge consequence in pollination. Lower pollination means lower fitness of both plants and bees. This is just a very small example, what could happen at a larger scale has a significant magnitude. A mismatch of phenological events, could thus lead to a loss of ecosystems functionality (Hoegh-Guldberg *et al*., 2018).

Species abundance and distribution are threatened by climate change. Indeed, 47% of the extinctions around the globe during the 20th could be attributed to climate change. Many species will not be only damaged by global warming itself, but also by highly invasive species that will establish in new suitable areas advantaged by temperature rise (Hoegh-Guldberg *et al*., 2018). Invasive species could be extremely competitive and threat the endemic species that are not use to more intense competition. Another important effect of climate change is species range shift in latitude and altitude. In the AR5 (Settele *et al*., 2014) it is shown a geographical move of 17 km poleward in latitude and 11 m up in altitude, as a result of global warming in the last decades. Some paleobiology studies reveal that during Mesozoic and Paleogene broadleaf forests exist at 85° latitude. Climatic conditions were characterised by warmer temperatures and higher concentrations of CO_2 (Royer *et al*., 2005). Therefore, some broadleaves and deciduous species could live at high latitudes if they could survive long periods winter of darkness.

Since carbon dioxide concentration in the atmosphere has been increasing from the pre-industrial era, reaching more than 410 ppm in 2020 (IPCC, 2018), biomass and carbon stocks have been increasing (Hoegh-Guldberg *et al*., 2018). Hence net primary productivity will increase, increasing the quantity of biomass. As a consequence, the decomposition rate will increase too. Major decomposition could drive to a negative carbon balance in forest ecosystems, due to a higher releasing than absorption of this latter in the atmosphere (Hoegh-Guldberg *et al*., 2018). Another important issue concerns the velocity of northward movement of temperature isolines and productivity isolines. Not all the ecosystems are able to respond effectively to an increasing of temperature and productivity. Namely many of the carbon sinks could not be ready to face such an increasing of atmospheric carbon dioxide. It happens especially at northern latitudes where minerals limitations, growing season length and growing season photosynthetic capacity restrict the productivity (Huang *et al*., 2017). Therefore, a general increasing of respiration rate of ecosystems associated with higher temperatures could convert boreal forests from carbon sink into carbon source, with a great impact on carbon balance at a global scale (Hadden and

Grelle, 2016). In addition, it is needed to consider that emissions will reach a peak and they will decline afterwards, causing a reverse tendency of carbon sink that was used to increasing concentrations of CO_2, they will more likely become carbon source (Jones *et al.*, 2016).

Forests play an important role in the Earth carbon cycle. They are, indeed, key carbon sinks where soil stores 44% of carbon, live biomass 42%, deadwood 8% and litter 5% (Pan *et al.*, 2011). Forests cover 30.08% of land area, meaning 4.06 billion hectares. Where 30% of them are considered primary forests, meaning natural regenerated forests of native tree species, with no visible signs of human activities (FAO and UNEP, 2020). These forests are sometimes referred to old-growth forests, between 15 and 800 years old (Pan *et al.*, 2011). These forests have an irreplaceable value, being the biggest sinks of carbon and biodiversity (FAO and UNEP, 2020). In regards of climate change mitigation, carbon storage is becoming one of the most important ecosystem services provided by forests (Fahey *et al.*, 2010). Nowadays, the integrity of primary forests is threatened by deforestation and extreme events. Earth surface covered by natural regenerated forest have been declining of 81 million hectares since 1990, due to deforestation for timber extraction, agricultural expansion and fires (FAO and UNEP, 2020). One of the biggest consequences of climate change is the increasing of frequency of intense extreme events like storms, wildfires, land degradation and pest outbreaks that can irreparably compromise ecosystems (Settele *et al.*, 2014). Disturbance on forest ecosystems have been increasing, causing a large-scale carbon loss (Seidl *et al.*, 2014). Globally, forest carbon stock decreased in the past thirty years, caused by an overall loss in forest area. Contrarily, it shows a positive trend in Europe, where it grew from 32 million tonnes in 1990 to 39 million tonnes in 2020 (FAO, 2020). Since forests are mainly used for production, meaning the 28 percent of total forest area (FAO, 2020), harvested wood used to store carbon in durable products or an alternative to fossil fuel, that postpone carbon emissions in atmosphere, plays a key role. The efficiency of carbon storage in forests is accomplish by management practices that include biomass conservation, type of forest and wood products produced (Fahey *et al.*, 2010). This means that good management practices can, indeed, make the difference in the carbon cycle equilibrium, diminishing greenhouse gases accumulation in the atmosphere (Seidl *et al.*, 2014; Fahey *et al.*, 2010). At the same time, biodiversity should also be included in management plans for carbon mitigation, finding good compromises in terms of cost-benefit (Anderson-Teixera, 2018).

There is still lot of uncertainty regarding carbon cycle under climate change (Settele *et al.*, 2014), and more literature is needed to better understand and model carbon changes with 1.5°C warming (Hoegh-Guldberg *et al.*, 2018). It is important then to understand which are the main physiological process involved in carbon absorption and release under global warming (Way and Yamori, 2013).

1.2 Photosynthesis and respiration

Photosynthesis and respiration are the key biochemical processes to understand plants carbon utilisation and therefore to study carbon balance of trees and forests. They are highly influenced by physical factors, like temperature and light. Under climate change scenario, it is fundamental to forecast fluxes of carbon (Way and Yamori, 2013).

1.2.1 Biochemical process

Photosynthesis is driven by the absorption of light by photosystem I and II, at different wavelength (respectively 700 and 680 nm) (Hogewoning *et al.*, 2012). In the thylakoid membranes

of the chloroplasts, the photosynthetic electron flow allows the production of O_2, NADPH and ATP (Trebst, 1974). Photons excites the chlorophylls located in the reaction centres. First the P680, or photosystem II, reaches an excited state and donate an electron to the pheophytin. Then, the P680, that was previously oxidized by light, is reduced by the oxygen evolving complex, that oxidize water into oxygen. Pheophytin transfers the electrons to the quinones that reduces the cytochrome b_6f, that in turn will transfer the electrons to the plastocyanin that reduces the P700, or photosystem I. This latter transfers the electron to a chlorophyll and a quinone, that transfers the electron to a sequence of iron-sulphur proteins. The ferredoxin accepts the electron and donates it to the ferredoxin-NADP-reductase that reduces the NADP in NADPH that will be used for the Calvin-Benson cycle (Blankenship and Prince, 1985).

Plants adopted different strategies to optimize photosynthetic reactions in different climate. Tree species used in the current experiment use C_3 photosynthesis, in which carbon dioxide is absorbed by the cells, it passes through a leaf boundary level and stomata. In this way it reaches the internal gas space and dissolve through the cell sap. Finally, it diffuses in the chloroplast where it is subjected to the Calvin-Benson cycle (O'Leary, 1988). In the chloroplast stroma, Rubisco catalyses the carboxylation of ribulose-1,5-bisphosphate (RuBP) obtaining a three carbons molecule: 3-phosphoglycerate (PGA). NADPH and ATP produced by the electron transport in thylakoid membranes are used to synthesised sugars and starch, and to regenerate RuBP (Yamori *et al*., 2013). Rubisco covers a crucial role in the carbon cycle, it is the most abundant enzyme on Earth and all the carbon that we eat and wear passed through its active site at least once (Cleland *et al*., 1998). Rubisco catalyses another reaction that, somehow, compromises the efficiency of the carboxylation pathway, it is, in fact, an oxidation way (Cleland *et al*., 1998). This process is called photorespiration. The catalyzation of both carboxilation and oxidation reactions is intrinsic of the active site of the enzyme. When Rubisco first appeared in non-oxygenic prokaryotes billions of years ago, photorespiration was not a significant process due to the lack of oxygen in the atmosphere (Bauwe *et al*., 2012). With the current atmosphere, characterised by a 20.95% of oxygen and 0.04% of carbon dioxide, photorespiration has a considerable effect on the photosynthetic yield (Moroney *et al*., 2013). Both compounds compete for the same site, and the rate of one or the other reaction is determined by the concentration of two molecules (Foyer *et al*., 2009). The way in which the Rubisco favours the carboxylase reaction is by stabilizing the six carbons compound that is formed before the cleavage in two molecules of 3-phospoglycerate (Moroney *et* al., 2013). At the actual concentration of O_2 in the atmosphere, every third molecule of RuBP becomes oxygenated in moderate temperature. The ratio increases at higher temperatures (Bauwe *et al*., 2012).

Photorespiration takes place in three different compartments: chloroplasts, peroxisome and mitochondrion. This time, two molecules of RuBP are oxidized in two molecules of phosphoglycolate and 2 molecules of phosphoglycerate. Phosphoglycerate will be used in the Calvin-Benson cycle, while the phosphoglycolate si dephosphated in glycolate by a phosphoglycolate phosphatase. Afterwards, the two glycolate molecules are transported from the chloroplast to the peroxisome, where a glycolate oxydase oxidases them with two molecules of molecular oxygen into glyoxylate. Glyoxylate is transformed in glycine through an aminotransferase, called glutamate glyoxylate aminotransferase. The two molecules of glycine generated in the peroxisome are then transformed into a serine by a glycine decarboxylase complex in the mitochondrion. During this reaction one molecule of carbon dioxide and one molecule of ammonia are lost. The serine is transferred back in the peroxisome, where a serine-glycolxylate aminotransferase converts it in hydroxypiruvate. The latter is then reduced in glycerate by the hydroxyporuvate reductase. The glycerate, is now phosphorylated in phosphoglycerate by a glycerate kinase in the chloroplast. Phosphoglycerate is finally used in the Calvin-Benson cycle (Moroney et al., 2013).

1.2.2 Temperature responses

Temperature is a key physical parameter that influences life on Earth. Therefore, all living organisms have developed signalling pathways to detect and react to temperature changes, preventing heat related damages (Mittler *et al.*, 2012). Plants adapt photosynthesis to temperature. In fact, photosynthetic rate can be described with an approximately parabolic curve, where the maximum represents the temperature optimum (T_{opt}). At both lower and higher temperature than T_{opt}, assimilation decreased until becoming null (Yamori and Hikosaka, 2013). The left side of the curve indicates that the temperature is too low to work at maximum potential. On the right side of the curve the assimilation declines because the Rubisco activase is thermo labile, thus the capacity of this latter to maintain Rubisco active declines with temperature. In addition, an increase of temperature leads to a decreasing of electron transport rate (Sharkey, 2005), hence to a lower production of ATP and NADPH. ATP is a needed to activate the Rubisco activase. If the availability of ATP is reduced by the elctron transport rate also the activation of Rubisco will be reduced (Dusenge *et al.*, 2019). A faster formation of dead-end products happened above the T_{opt}, slowing down the activity of Rubisco (Salvucci and Crafts-Brandner, 2004). The carboxylation rate of RuBP is also linked to the change in T_{opt} (Yamori and Hikosaka, 2013).

Temperature warming can increase the oxidation pathway of Rubisco. The specificity of Rubisco for O_2 increases and the solubility of molecular oxygen decreases slower than the one of carbon dioxide, concerning a major availability of O_2, so a preference for the oxidation (Dusenge *et al.*, 2019). Heat stress affect the stability of various proteins, membranes RNA species and cytoskeleton structures, that can change the efficiency of the photosynthetic process (Mittler *et al.*, 2012). Hence, plants developed adjustment strategies to low and higher temperature, so that, they can maximize the photosynthetic rate to the growth temperature (Yamori and Hikosaka, 2013). As consequence, plants acclimated to lower temperature will have a lower T_{opt} than the one acclimated to higher temperature.Not all the plants respond the same way to temperature stress. In fact, some species can adapt better to temperature changes, for instance cold-tolerant plants can lower T_{opt} more than cold-sensitive plants (Yamori *et al.*, 2010).

Rubisco, RuBP and inorganic phosphate (P_i) are key targets for photosynthesis regulation. At low temperature some species can show an increasing in sugar biosynthesis and P_i availability that contrasts the much higher presence of phosphorylated compounds (Strand *et al.*, 1999). At the same time, the regeneration and carboxylation of RuBP rate are increased (Hikosaka *et al.*, 2005). By contrast, mechanisms of temperature responses at high temperature are not completely understood (Yamori and Hikosaka, 2013). Long term thermal responses of photosynthesis and respiration are still not completely clarified. Despite, it is known that temperature acclimation of photosynthesis is often linked with respiration, thus the need to study them together. Lot more efforts are needed to clarify whether respiration can overpass assimilation under climate change scenario (Dusenge *et al.*, 2019).

1.2.3 Light responses

Sunlight is the primary source for the photosynthetic pathway, but it also controls many developmental and physiological responses (Kong *et al.*, 2016). Plants are able to adapt to light in order to regulate photosynthesis and to avoid light stress damages. Therefore, there are both short-term and long-term responses to light. First ones concern the daily variation of light, due to sun flecks

or diurnal change of irradiance. Second ones, instead, relate to gene expression that regulates leaf structure, composition of chlorophylls and carotenoids, number of rection centres, and size of photosystem antennas (Bukhov, 2004). Light sensible receptors transform light signals in biochemical responses, like protein-protein interactions. Specifically, phytochromes receive red/red-far light signals, whereas Cryptochrome and Phototropin respond to blue light signals (Kong *et al*, 2016).

Since not all the light received can be used for photosynthesis, plants develop different strategies to manage excess excitation energy (EEE). First of all, some of the light is dissipated through fluorescence or through heat by the non-photochemical quenching (NPQ). Secondly, there is a transfer of excessive electrons to oxygen. It generates reactive oxygen species (ROS), that can create cellular damage and activate stress responses in the cells (Karpinski *et al.*, 2012). Hence, the generation of ROS due to light stress is called photooxidative stress. Cells developed mechanisms against photooxidative stress, namely antioxidative systems placed in the chloroplasts. However, ROS are an important alarm to modify metabolism and gene expression in response to adverse environmental conditions (Foyer *et al.*, 1994).

Photosynthesis is primarily influenced by the quantity of light. Plants plasticity allows them to regulate due to irradiance. Plants form, physiology and resource allocations are shaped by the amount of light that the plants generally receive (Givnish, 1988). The plasticity of different species is a key topic to understand forests dynamic. There are, indeed, plants that are likely to better adapt to shade environments than others (Valladares and Niinemets, 2008).

To understand the capacity and the way plants perform under different light levels, it is relevant to experiment light response curves. Light response curves explain net photosynthesis (P_N), meaning CO_2 assimilation rate, as a function of the photosynthetic photon flux density (I). The curve is performed starting from darkness to high levels of light (from 0 µmol (photons) m^{-2} s^{-1} to ca. 2000 µmol (photons) m^{-2} s^{-1}). The first part of the curve is very steep, it is characterized by a rapid increase of P_N from dark respiration (R_D) until a level at which the assimilated CO_2 is equal to the respired one. Hence, the I value at which P_N is equal to zero is called Light Compensation Point (LCP). Beyond the LCP, the curve assumes a linear trend that is called '*Maximum Quantum Yield*'. The latter represents the slope of this trait and it ends in a non-linear trend, that is described by a convexity factor (θ), as well as the $\delta P_N/\delta I$ ratio. Afterwards, the curve reaches a plateau, where the photon flux saturates the electron chain and the P_N gets to the maximum rate (P_{gmax}). Sometimes, a phenomenon called photoinhibition could occur, so a decrease in P_N is seen in the curve (Lobo *et al.*, 2013).

1.3 Winter dormancy

Trees are subjected to an annual rhythmicity in which they alternate summer periods of growth and winter periods of dormancy (Havranek and Tranquillini, 1995). In temperate and boreal zones, it is important for plants to maintain the synchrony of growth, winter dormancy and frost hardiness with the seasonal changes (Olsen, 2010). Dormancy is defined as the inability to initiate growth from meristems or other organs and cells with the capacity to resume growth under favourable conditions (Rohde and Bhalerao 2007). Dormancy is prevalently controlled by daylight length. The shortening of photoperiod during autumn is one of the major drivers of winter dormancy (Olsen, 2010). But temperature plays also a key role in some species, for example cessation of growth can be induced in Norway spruce during long photoperiod if the night temperature decreases (Olsen, 2010). What allows trees to survive cold winters without suffering cold temperature is a substantial change in the physiology and composition of the cells. During the winter dormancy, resting buds can be seen, as well as no elongation. At the cellular level, the metabolic activity is extremely reduced and there is a change in the cytoplasmatic structure to

survive frost and dissection (Havranek and Tranquillini, 1995). Gene expression does not change significantly during winter, meaning that the maintenance of a rest condition is not transcriptional dependent. Despite, hormones play an important role in the regulation of dormancy. For example, the gibberellins levels are down-regulated in angiosperms and conifers due to the shortening of photoperiod, auxin and ethylene seem to play determinant roles in the switch from dormancy to growth or vice versa, but the functions are still unclear (Olsen 2010).

In evergreen trees, photosynthetic activity during winter is low (Bourdou, 1959; Havranek and Tranquillini, 1995). Nonetheless, chlorophyll content in needles is significantly reduced (Hansen, 1996). But, under climate change scenario, mild winters can affect the exit from dormancy, favouring the release of vegetative buds during winter (Havranek and Tranquillini, 1995, Harsen, 1996). In addition, especially plants in northern latitudes are more subjected to spring frost damages. If the release of vegetative buds starts too early, when the occurrence of frost is still likely to happen, they can be strongly damaged (Havranek and Tranquillini, 1995, Fu *et al*., 2014).

1.3.1 Carbon balance during winter

Evergreen trees maintain the photosynthetic apparatus active during winter because it resists to frost temperatures. Therefore, the maintaining of leaves with the photosynthetic apparatus during winter allows plants to make photosynthesis if the temperatures are sufficiently high (Wyka *et al*., 2014). However, the net photosynthesis during cold months is strongly influenced by the respiration rate. It is thus important to understand which are the main factors that intensify and decrease levels of respiration (Medlyn *et al*., 2005). Temperature and photosynthetic active radiation influence the gross primary production of plants, meaning photosynthetic and respiration rates. The level of carbon loss is, indeed, strictly related with climatic conditions (Hansen *et al*., 1996; Medlyn *et al*., 2005). In the climate change scenario, where mild winters can occur more frequently, the respiration rate needs to be considered in the annual carbon balance of the plant. Respiration, indeed, consumes between 54% and 71% of the annual net photosynthesis (Ryan *et al*., 1997). A study of Hansen *et al*., (1996) revealed carbon allocation during a whole year in Scot pines, using radio $^{14}CO_2$. In this way they could control the distribution of carbon in pines. It appears that more than 50% of the radio-carbon fixed at the beginning of the experiment, during January, was respired in the first week. In addition, the majority of carbon dioxide absorbed was maintained in the needles as sucrose during cold months. Increasing the concentration of sugars in the needles contributes to the frost damage resistance, although a part of these sugars can be respired with mild temperatures (Ögren, 1984; Strimbeck, 2008).

So, considering that the oxygenation rate of Rubisco increases faster than the carboxylation with rising temperatures (Farquhar *et al*., 1980), it is very important to understand where carbon is allocated and how much of it is respired under different climatic conditions to better face climate change. Therefore, quantifying the effect of each climatic variable could be very useful to depict the global amount of respiration during winter months (Medlyn *et al*., 2005).

1.4 Phenology

Phenology is defined as the study of life cycle events of animals or plants, as influenced by the environment (Cleland *et al*., 2007). Day length, temperature and winter chilling are the major drivers of phenology. Photoperiod controls the winter events, such as the appearance of winter buds, leaf

abscission meristem and freezing resistance. Besides, it regulates the exit from dormancy and the consequent spring events (Körner and Basler, 2010). Temperature influences the beginning of growing season at different levels, depending on the species (Körner and Basler, 2010). The increasing of temperature, which ecosystems are experiencing with climate change, is advancing plants spring events (Cleland *et al*., 2007; Penuelas *et al*., 2009; Körner and Basler, 2010). Global warming has been advancing the phenological spring events of 2.5 days per decade (Körner and Basler, 2010). The advance of spring is linked with a delay in the beginning of autumn phenology, causing a general increasing on the length of the growing season. It is important to understand whether this change can affect the absorption of CO_2, with a substantial increasing in the fixation rate in plants, as well as an increase of the GMST due to an earlier green cover of the ground and a reduction of the albedo (Penuelas *et al*., 2009).

Plant phenology has a key role in the regulation of ecosystem phenology (Chuine and Régnière, 2017). There are, indeed, optimal time windows for the supply of food resources among organisms. Since plants are primary producers, they cover the basics regulation of the trophic chain. Hence, consumer species demands should be the highest when the offer is the highest. But, under global warming, the shift of phenology at different scales in different organisms is mismatching the phenological events, causing for example damages at populations level due to a mistime of demand-supply of resources. It means that the fitness of many populations will be affected by matching the phenological events needed for their survival (Visser and Gienapp, 2019).

Phenological models predict and evidence the trend of phenological events. Many different functions can be used to describe response of plants to temperature and daylength. There are especially many studies that use a non-linear monotonic function, meaning a sigmoid curve, that will be taken into account in the context of this project as well (Chuine and Régnière, 2017). Predictability of phenological events is crucial to understand primary productivity and gas exchange under climate change scenario. Nonetheless, it also helps to better understand population dynamics and species interaction. Therefore, a selection of the cultivars that will better adapt to global warming without affecting the ecosystem services offered by a specific ecosystem, will play a key role for future climate change mitigation (Cleland *et al*., 2007).

1.5 Study case of this project

The project wants to explore how mild winters are influencing plants performances during winter and growing season. Therefore, following paragraphs will explore the Danish environmental conditions and forests future projections under climate change. Finally, the species that were chosen for this experiment will be presented and analysed in the terms of the aim of the experiment under a possible climate change scenario.

1.5.1 Danish climate

Denmark has a relatively warm climate comparing with other regions located at the same latitude. The warm North Atlantic current that comes from the east coast of United States after being warmed up in the Caribbean is the main reason of a more temperate climate. However, the environment is strongly influenced by the position. Denamrk is surrounded by water, as well as by the continental lands in the south, namely the north-central Europe. Therefore, the weather changes a lot with the direction of the wind, switching from temperate to continental and vice versa. Mean temperature recorded between 1980 and 2010 was 8.3°C, whereas it increased in the decade of 2006-2015 with an average of 8.9°C. The

effect of global warming had, thus, shown an overall temperature rise of 1.5°C from 1870s (Cappelen, 2020). Precipitation and hours of sun have an important variation year by year. In the period between 1980 and 2010 the annual average of rain was 746 mm and the recorded hours of sun were in average 1,574. Global warming led to an increase of 100 mm per year of rain in the last hundred and fifty years and to a general increase of hours of sun from 1980s comparing with the rest of the century (Cappelen, 2020). Wind speed is strictly dependent on the position. It is, indeed, stronger in the coastal region than in the inland. Most of the storms and hurricanes occur during winter months. There are not significant changes in the wind climate from the mid of 19th century (Cappelen, 2020).

It is already well known that global warming will be stronger at northern latitudes (IPCC, 2013). The main reason is the melting of arctic ice perennial covers that will diminish the albedo effect. Meaning that the energy that was reflected by the white surface of ice, will be more and more absorbed by the black cover of ground that remains after the ice melting, causing an increase of GMST. Denmark had already recorded the highest temperature decade of the last hundred years in between 2007-2016, where the temperature was 0.6°C higher than the average between 1961-1990 (Stendel, 2018). An overall increase of air temperature leads to a greater capacity of air to carry a larger amount of water. If there is more water in the air, there is also a major energy in it. Meaning that the power with which water is released is stronger (Christensen, 2018). Therefore, northern hemisphere, and especially northern latitudes will experience an increase of precipitation (Stendel, 2018), with more extreme rainfalls (Christensen, 2018). In addition, extreme events will occur more frequently during summer, alternating periods of heavy rain with periods of drought. It means that there will be an unequal distribution of rain that will have huge impacts on the ecology of Danish ecosystems (Christensen, 2018). Nonetheless, temperature rise will reduce cold days (IPCC, 2013), meaning less frost days (Stendel, 2018). What Denamrk will experience in future decades are wetter, milder and greyer winters, with more rain. One of the main consequences is the saturation of soils, followed by a smaller evaporation during cold months (Chirstensen, 2018).

1.5.2 Danish forests

Denmark is characterized by mesophytic deciduous broadleaved and coniferous-broadleaved forests (EEA, 2006). Forests cover 628.44 hectares of the whole land (4,199 thousand hectares), meaning 14.97%. Climatic domain of the latter is temperate (FRA, 2020). They have economic, landscape and recreational value (Olesen, 2018). More than 20% of the forests are old forests, and 17% are recently regenerated forests. (FRA, 2020). Denmark, specifically, designated 80 percent of its forest for production, ranking itself as the world second country for percentage of forests used for this purpose (FAO,202). Most spread native species are *Fagus sylvatica* and *Quercus robur*, that are respectively marked as the first and the second in terms of volume (FRA, 2020). However. most of the forest land is covered by conifers that were introduced 200-300 years ago for production purposes. Evergreen conifers are, indeed, more profitable trees than deciduous ones because of their quick growth (Environmental Protection Agency [EPA], n.d.). Among the conifers *Picea abies* (Norway spruce) is the most common, covering 19% of total forest area (FRA, 2020). However, tree composition is likely to change under global warming. Many environmental hazards, like fires, storms, diseases and drought, will increase their frequency in time, meaning that forest will be subjected to a shift in species that will better adapt to these conditions (Olesen, 2018).

1.5.3 Species selected in the experiment

Five species were selected for the experiment. Namely *Picea abies* (Norway spruce), *Abies alba* (Silver fir), *Larix X eurolepis* (Hybrid larch), *Quercus robur* (Peduncolate oak), and *Fagus sylvatica* (European beech). Hence, there are two evergreen conifers, one deciduous conifer and two deciduous broadleaf trees.

Picea abies is the most common tree in Denmark, and it was introduced 250 years ago (Larsen *et al*., 2005). It seems to enhance its growth rate with an increasing in temperature and carbon dioxide concentration, meaning that it could be advantaged by global warming (Kellomäki and Kolström, 1994; Elizondo *et al*., 2006; Jansson *et al*., 2008). However, it is important to understand how the geography of the place will influence the resistance of the plants. Regional changes are important to be considered to have more precise projections of the future of the spruce under climate change (Vacek *et al*., 2019).

Abies alba has high resistance to wind and airborne salt (Hansen and Larsen, 2004). It is a very important species for ecological and socioeconomical reasons, it offers recreation landscapes, biodiversity and protection from erosion (Vitasse *et al*., 2019). How the species will react to climate change is still unclear (Gazol *et al*., 2015; Vitasse *et al*., 2019). Paleological studies reveal, indeed, that it was distributed in areas subjected to much warmer temperatures. Nevertheless, other studies forecast a general decline of its spread due to climate change (Vitasse *et al*., 2019). It seems to be declining in areas were drought occurs more frequently (Gazol *et al*., 2015). However, many studies show a better resistance than *Picea abies* in the future scenarios (Vitasse *et al*., 2019).

Larix X eurolepis is a hybrid species generated by the cross of *Larix decidua* **(**European larch) and *Larix kaempferi* (Japanese larch). It was included in the experiment because it seems a good complement of *Picea abies* in commercial forestry, and as an example of deciduous conifer. It shows, indeed, a great yield of growth (Larsson-Stern, 2003).

Quercus robur is the second most spread native species in Denmark. It shows very different responses under climate change scenarios among populations. Hence, there might not be a linear pattern of feedbacks (Morin *et al*., 2010). However, a study by Huang *et al.,* (2017), evidences that *Quercus robur* is the only species which will benefit from the predicted climate changes in Denmark, considering a small reaction to varying precipitation and temperature during the growing season. Pedunculate oak is, indeed, considered a frost and drought tolerant plant (Larsen, *et al.*, 2005).

Fagus sylvatica is an important economic and ecological resource in Europe, and especially in Denmark, being the most abundant native species. It is thus important to understand the effects of climate change on it (Prislan *et al*., 2019; FRA, 2020). Responses of this species to global warming are strictly related to the regional characteristics, meaning that there is a location dependency to consider in the future projections (Kramer *et al*., 2010). It seems that one of the biggest damages will be caused by drought during the growing season (Geßler *et al*., 2007; Prislan *et al*., 2019). Beech trees seem to be, overall, negatively affected by climate change, resulting in a spread decline over Europe (Dulamsuren *et al.*, 2017).

1.6 Objectives

Under the climate change scenarios temperatures are expected to rise much more strongly in high northern latitudes compared to the average global warming (Orlowsky and Seneviratne, 2012; Collins *et al*., 2013; IPCC, 2013). Higher temperatures increase plants respiration rate (Atkin & Tjoelker, 2003; King *et al*., 2006) that could lead to a significant CO_2 concentration rise in the atmosphere (King et al., 2006). The Printz (1933) hypothesis suggests that respiration will exceed photosynthesis during mild winters, causing a negative carbon balance. Moreover, warmer temperatures during winter could also affect plants phenology and growth (Huang et al., 2017). On the other hand, winters will become greyer with an increased cloud cover (Chirstensen, 2018) and shaded plants show a lower compensation point, but also a lower respiration rate (Leverenz, 1995). In addition, considering the biome shift (Settele *et al*., 2014) from the paleobiological point of view, it is important to understand if some broadleaf plants are able to adapt to darkness during winter (Royer *et al*., 2005).

Some of the most significant plants for Danish ecosystem will be tested in a mild winter scenario with different light and temperature exposures. Namely we want to explore plants performances under different temperature treatments through an analysis of temperature and light curves. We want to study the respiration of plants during winter, exploring respiration in the dark and the correlation with temperature. In addition, we want to understand the diurnal performances of plants, calculating the carbon uptake under different temperature and light treatments. Finally, we want to see if some species would be affected by darkness during winter, considering latitude shifting of species. Afterwards, we want to point out if there is an effect of temperature and light occurred during winter on plant performance, growth and phenology.

2. Materials and Methods

2.1 Plant material

488 seedlings were used for the experiments: 76 *Abies alba*, 100 *Fagus sylvatica*, 97 *Larix X eurolepis*, 116 *Picea abies* and 99 *Quercus robus*. Plants were firstly potted in 20 cm diameter pots, and then repotted in a 35 cm diameter pot before the growing season, on March 2020. During potting and repotting two Osmocote fertilizer tabs were added to each pot. Selected plants had different ages and grew in different ways. At the moment of first potting in August 2019, *Abies alba* was four years old and transplanted bare-rooted, *Picea abies* was one and half years old grown in Jiffy, *Larix X eurolepis* was one year old grown in Jiffy, *Quercus robur* was two years old transplanted bare-rooted, and *F. sylvatica* was three years old and already potted (it was previously used for autumn temperatures experiment in 2018, information about the previous treatment are trackable). Plants origin was also tracked: *Abies alba* – FP242 Denmark, *Picea abies* – FP635 Denmark, *Larix X eurolepis* – FP203 Denmark, *Quercus robur* – Elsendrop, Netherlands, and *Fagus sylvatica* – FP849 Denmark. Seedlings were placed in outdoor ambient conditions from November 2019 until January 2020. After an initial screening, removing unhealthy plants, plants were selected and moved to the plots on 3rd of January. Selection was done by dividing all the species in two groups of taller and shorter plants. After that, two of each group were selected randomly and moved to an arbitrary plot.

2.2 Experimental designs

The experiments took place in the Arboretum, Horsholm (55°51'57.36"N - 12°30'30.64"E). Plants were subjected to different light and temperature treatments for one month, from the 7th of January until the 7th of February 2020. Outdoor and indoor plots were set respectively in a 500 m^2 yard and in four greenhouses nearby the yard [**Fig. 2.1**]. The outdoor experimental design ensured that the natural winter environmental conditions (e.g. humidity, precipitation and wind) were not altered for both control and heated plots, but the temperature treatment was then limited by heaters capacity in a such cold environment and with limited energy costs. On the other hand, the indoor treatments didn't keep the natural ambient conditions (plants were not subjected to wind and precipitations), but temperature treatments could be more effective, generating a major gap between control and indoor plots. Therefore, two different experimental designs were set up for outdoor and indoor experiments, following space and plants availability. Plants located in the outdoor plots had shoots of the first whorl set at the same distance (approximately 80 cm) from the heaters to receive an equal amount of heat. Heaters were turned on during the morning of 7th of January, date that establish the beginning of experimental month of light and temperature treatment. The treatment ended on the 7th of February. Afterwards, all the plants were moved to two squared plots and placed in random position in the colder greenhouse (indoor seedlings) and outside (outdoor seedlings).

2.2.1 Outdoor experiment

Two different temperatures and two different light treatments were designed for this experiment. Eight 12 m^2 hexagonal plots were placed in the yard [**Fig. 2.2**]. Four plots were heated through six infrared non-glowing heaters each set at two meters height from the ground, to heat up the canopy inside the plots, aiming to obtain the highest temperature (+4°C compared to the control plots) at 80 cm from the ground. Four plots functioned as control with natural environmental conditions, thus six coloured wooden boards were mounted in each plot, simulating the heaters. In addition, each plot was subjected

to two different light exposures. Hence, half of the plants in each plot were covered by a net that traps 60% of light, and the other half was exposed to ambient light. Nets were fixed on a wooden structure built inside each plot. The structure was keeping the net between the plants and the heaters. Plots were named with numbers, from '1' to '8', pair numbers represented heated plots and odd numbers the control ones. Light treatments were named 'Light' to refer to ambient light, and 'Shade' to refer to shaded plants. In this way, four different treatments were included: 1) ambient temperature and ambient light, 2) ambient temperature and shaded, 3) heated and ambient light, and 4) heated and shaded [**Fig. 2.3**]. Forty plants were placed in each plot with 20 of them exposed to ambient light and 20 set in shadow. In particular, seedlings were divided in each plot as follows: 6 *Abies alba*, 8 *Fagus sylvatica*, 8 *Larix X eurolepis*, 10 *Picea abies* and 8 *Quercus robur*. Therefore, there were 12 *Abies alba*, 16 *Fagus sylvatica*, 16 *Larix X eurolepis*, 20 *Picea abies* and 16 *Quercus robur* per treatment, resulting in 80 seedlings per treatment and 320 plants in total. The seedlings set in the plots were surrounded by side plants to limit the wind action and to reach a higher temperature.

Figure 2.1 – **Arboretum map.** The Arboretum is located at Horsholm (55°51'57.36"N - 12°30'30.64"E). On the top left the yard used to set up the plots for the outdoor experiment. Greenhouses are evidenced with different colours the evidence temperature treatments.

2.2.2 Indoor experiment

The indoor experiment was established in 4 greenhouses and a control plot outside. Temperatures in greenhouses were adjusted by heaters and had different mean temperatures: the warmest (*G13*) was 13°C, the warm (*G11*) was 10.6 °C, the medium (*G9*) was 8.7 °C, the lowest (*G6*) was 5.8 °C and the outside plot 1 had ambient temperature [**Fig. 2.4**]. Half of the plants were exposed to ambient light and half to a dark treatment (PAR = 0 µmol m-2 s-1). The latter was realized with a black plastic cover placed all around the seedlings. Indoor treatments were called 'Dark' for plants set in darkness and 'Light' for plant exposed to normal light. 38 or 39 seedlings were placed in each greenhouse plot, and 13 in darkness outside (plot 1 outdoor was used as ambient light control for this experiment). The number of seedlings in the greenhouses were divided as follows: 38 plants in *G13* and *G11*, 39 plants in *G9* and *G6*, and 13 plants in dark control outdoor [**Tab. 2.1**]

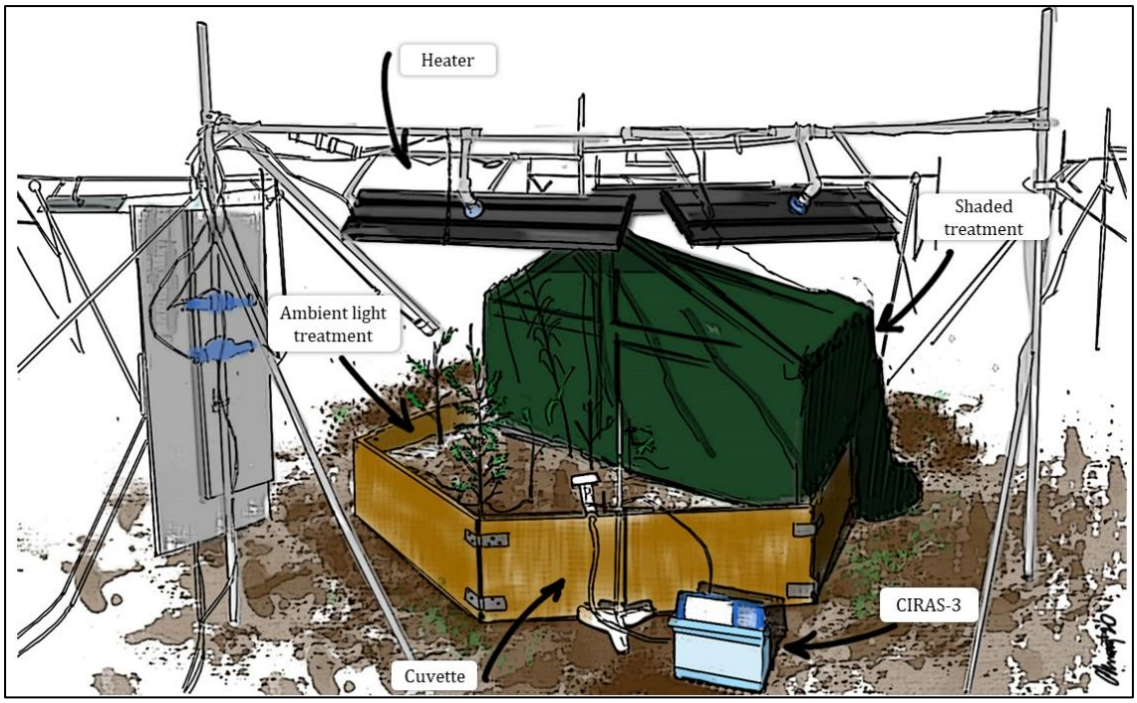


Figure 2.2 – **Outdoor plot set up**. Each plot had an ambient light and shaded treatment. Gas exchange measurements were done *in loco*. This picture shows one heated plot. Control plots had the same set up but the heaters were replaced with black wooden board.

Table 2.1 – **Species divided by treatment**. Plants distribution in both outdoor and indoor experiment.

		Abies alba	*Picea abies*	*Larix X eurolepis*	*Quercus robur*	*Fagus sylvatica*	Tot. per plot
Outdoor experiment	**Control shade**	12	20	16	16	16	80
	Control light	12	20	16	16	16	80
	Heated shade	12	20	16	16	16	80
	Heated light	12	20	16	16	16	80
Indoor experiment	**Dark out**	3	4	0	3	4	14
	G13	6	8	8	8	8	38
	G11	6	8	8	8	8	38
	G9	6	8	9	8	8	39
	G6	7	8	8	8	8	39
	Tot. per species	76	116	97	99	100	488

2.3 Environmental conditions

Environmental conditions, specifically temperature, light, precipitation, wind and humidity, were monitored outside during the whole experiment thanks to a HOBO meteorological station placed among the eight plots. Wind was also monitored with CR1000X logger (Campbell Scientific, US). Millimetres of rain dropped every hour during the experimental month, were downloaded from the Danish Meteorological Institute web site. In addition, all the outside plots had their own thermometer, namely an Apogee infrared temperature sensor, placed above the canopy measuring temperature at the canopy surface (Apogee Instruments, Logan, UT). Thermometers were connected to a computer able to record

temperature data every minute. The computer was able to detect temperature input signals from control and heated plots thermometers and to turn on and off the heaters in the heated plots to maintain 4°C of difference between the control and heated plots [**Fig. 2.5**].

HOBO soil temperature sensors were set in eight pots, one per species (excluding beech), four in control and four in heated plots at 10 cm depth from the top of the soil. They were first placed in plot 3 and 4, and they were moved to plots 7 and 6 after two weeks. Data recorded by HOBO loggers were downloaded regularly using the HOBOware software.

Temperature and PAR were monitored for the indoor experiment through loggers (GMR STRUMENTI SAS, Scandicci, Italy) linked to the sensors set in each greenhouse and control outside. Data were recorded every hour and downloaded trough WSN acquisition suite (Florence Engineering srl, Firenze, Italy).

2.4 Gas exchange measurement

To understand the respiration and photosynthetic rate under different treatments and individuals, gas exchange measurements were performed on *Picea abies* and *Abies alba* during the experimental month. Gas exchanges were measured with CIRAS-3 portable photosynthetic system (PP Systems, Amsbury, MA, USA) connected to a standard 10 cm^2 chamber with transparent top. Values of CO_2 fixation rate (A) and stomatal conductance to water vapour (gs) were recorded after the machine reached stable values, which approximately took 2-5 minutes according to the conditions. CO_2 reference concentration set was 400 $\mu mol\ mol^{-1}$, and relative humidity of the air was approximately 70% of ambient. All gas exchange parameters were initially calculated with set leaf area 10 cm^2.

All measured branches were collected after the end of the treatments (7th February), in order to measure the photosynthetic surface of each individual and to normalize collected data. Needles from these branches were, indeed, individually put on see-through tape to avoid overlap. Prepared tapes with needles were then scanned with Epson Expression 11000XL scanner (Japan) at 600DPI. Images were further analysed with ImageJ (LOCI, USA) and total leaf area per branch was assessed.

The timeline for the experiment was divided in four weeks, and every week two types of measurements were done on both the indoor and outside plants: (1) ambient light measurements and (2) diurnal curves. In addition, temperature and light curves were carried out on different individuals during the experiment.

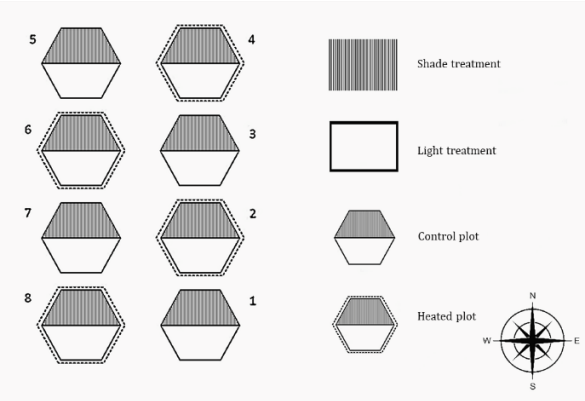


Figure 2.3 – **Plots of the outdoor experiment.** Plots are divided by temperature and light treatment.

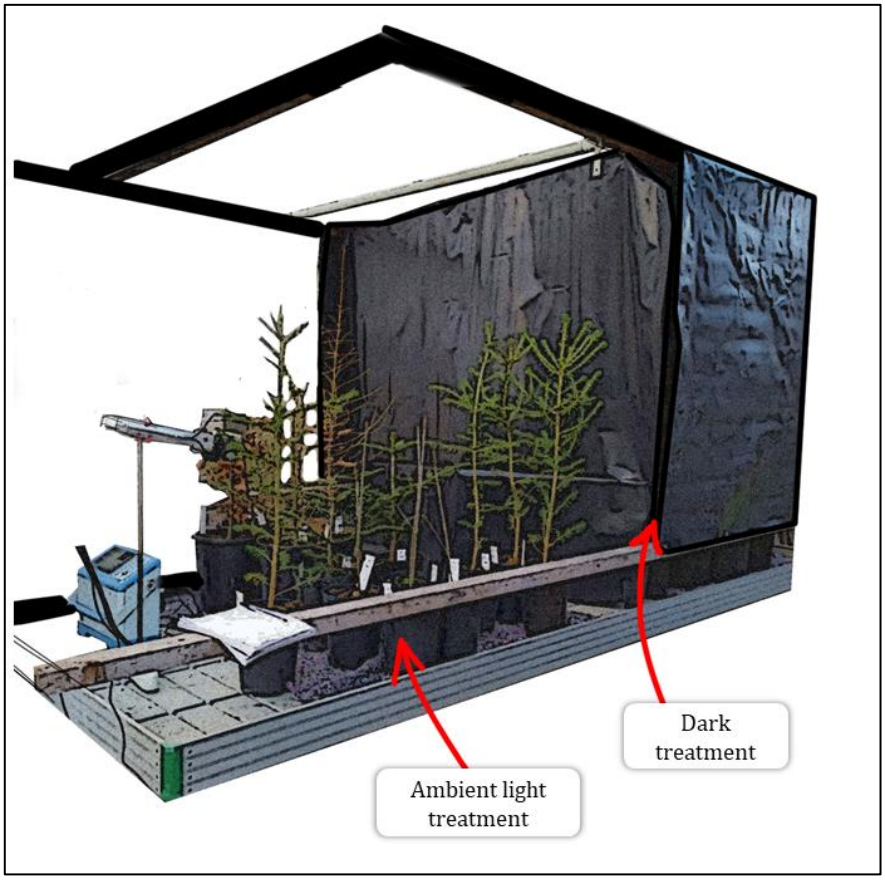


Figure 2.4 – **Indoor plot set up**. Indoor plots were placed in the four greenhouses setting up a total dark and an ambient light treatment. Gas exchanges were measured *in loco*.

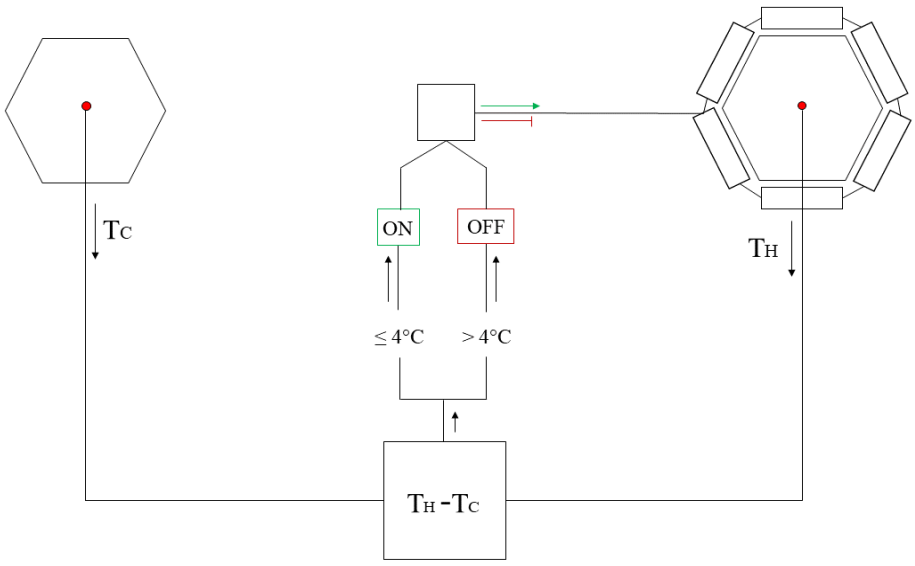


Figure 2.5 – Temperature control mechanism for heated and control plots – outdoor experiment. Apogee infrared sensors in the control plot (top left) and in the heated plot (top right) send two temperature signals, respectively Tc and T_H, to the central computer (down centre). The central computer calculates the difference between T_H and T_C. If the difference is less than +4°C the computer sends an output signals that turns on the heaters in the heated plot. In opposite case, the computer sends a signal that turns them off.

2.4.1 Ambient light

Ambient light measurements were done once a week for four weeks. All the plants in each plot were measured one time under ambient conditions. Data were recorded with different PAR, so that a wide spectrum of performance under different conditions was obtained [**Fig. 2.6**].

Figure 2.6 – **Gas exchange measurements.** They were performed with CIRAS-3 portable photosynthetic system (PP Systems, Amsbury, MA, USA). On the left a measurement performed with using PLC3 Universal LED Light Unit (RGBW) (PP Systems, Amsbury, MA, USA), on the right some preliminary tests with the machine using ambient light.

2.4.2 Diurnal curves

Diurnal curves were carried out once a week for four weeks on both indoor and outdoor plots. Diurnal curves consisted in five cycles of measurements with an approximative range of two hours each. Measurements were starting before the sunrise and ending after the sunset, in order to measure both respiration and net photosynthesis. One individual per plot per treatment was randomly selected during the first cycle and measured during the whole day [**Tab. 2.2**]. Selected individuals were changed every week, in order to have data from as many individuals as possible. In addition, the order in which plots were measured was changing every week (e.g. cycle measurements started at 6:00 am from plot 1 on the first week, and from plot 8 on the second week). Every individual was measured *in loco*. Both sunny and cloudy days were chosen to have different levels of PAR and temperature during the cycles. Daylight was approximately between 7-8 hours from the beginning to the end of the experimental month. Hence, sunrise was approximately after 8:00, and sunset after 16:00, so that the first and the fifth cycle of measurement were taken in almost darkness.

Table 2.2 – **Time scheduled for diurnal curves cycles.** Table shows the five cycles of measurements performed during the day, the first and the last cycle were performed in darkness.

Cycle	Time
1	06:00 – 08:00
2	09:30 – 11:30
3	12:00 – 14:00
4	14:30 – 16:00
5	16:30 – 18:30

2.4.3 Temperature curves

Temperature curves were performed on *Picea abies* and *Abies alba* indoor. Different PAR levels were obtained thanks to PLC3 Universal LED Light Unit (RGBW) (PP Systems, Amsbury, MA, USA). Measurements were carried out in the warmest and coldest greenhouses (*G13* and *G6*), testing the plants at a constant PAR of 100 µmol m^{-2} s^{-1} and 200 µmol m^{-2} s^{-1}. Levels of PAR with which temperature curves were performed were chosen according to the mean level of light that occurs during winter in Denmark. Temperature was varied from 3-4°C to 25°C in five steps [**Tab. 2.3**].

2.4.4 Light curves

Light curves were performed in the four greenhouses on plants exposed to ambient light. Two individuals per greenhouse per species were randomly chosen among *Picea abies* and *Abies alba*. Curves were obtained using PLC3 Universal LED Light Unit (RGBW) (PP Systems, Amsbury, MA, USA), that allows to change the amount of light in the leaf chamber. PAR levels were increased from 0 µmol m^{-2} s^{-1} to 1000 µmol m^{-2} s^{-1}. Temperature was set at the ambient temperature of the analysed greenhouse [**Tab. 2.3**].

2.5 Plants dimension measurement

Seedlings dimensions, diameter and height, were measured before the 7th of January and after the 16th of June, therefore before the experimental month and after the full development of new leaves. A ruler was used to quantify the height of the trunk and a caliber was used to measure the diameter at 8 cm from the ground. This point was marked in order to perform further measurements in the same position.

Table 2.3 – PAR and temperature levels set for temperature and light curves in different greenhouses. Table shows number of selected individuals for light and temperature curves, with light and temperature settings we used for each one.

	Individuals and greenhouses	**Set PARs**	**Set Temperatures**
Temperature curves	1 per species per greenhouse (G13 – G6) (no dark treatment plants were used)	(100, 200) µmol m^{-2} s^{-1}	(3, 12, 15, 19, 25) °C
Light curves	2 per species per greenhouse (G13-G11-G9-G6) (no dark treatment plants were used)	(0, 20, 40, 60, 80, 100, 150, 200, 500, 1000) µmol m^{-2} s^{-1}	G13 = 14°C G11-G9 = 10°C G6 = 5°C

2.6 Phenology

The phenological development of plants was assessed during the growing season. Since some of the buds were showing a green colour already during the treatment, data were collected from 3rd of February [**Fig. 2.7**]. The monitoring continued with a frequency of one campaign every second week until the growing season started. It took place once a week since then. Phenology was monitored for all the 488 plants. Buds were evaluated using two different scales, one referred to angiosperms and the other to conifers [**Tab. 2.4**]. A score was given to the buds according to the appearance of the majority of the buds on the up-quarter part of the seedling but excluding the buds at the top of the shoot.

Figure 2.7 Phenological measurements and set up. (**A**) picture of the first bud breaking in *Larix X eurolepis*, (**B**) seedlings of indoor experiment moved in *G6*, and (**C**) seedlings of outdoor experiment moved in one unique plot outside.

Table 2.4 – Phenological scores for Angiosperms and conifers. Table shows the scores that were given to angiosperms on the left and scores used to evaluate conifers phenology on the right.

Angiosperms

Score	Stage	Score	Stage
0	Winter	5	Very small leaves, just escaped from buds
1	Buds swelling	6	Small leaves at the start of expansion
2	Buds green	7	Leaves in an advanced stage of expansion
3	Buds breaking	8	Leaves have reached final size, but still appear unhardened, spring-like (bright green).
4	Leaves partly out of buds	9	Fully developed, fully hardened leaves.

Conifers

Score	Stage
0	Bud in winter condition
1	Bud slowly starting to swell, no green is seen
2	Bud swollen, some green is seen, but bud scales are still covering bud
3	Bud scales dropped, bud not elongating or only very little
4	Shoot started to elongate, needles brush-like forward pointing (<3cm)
5	Shoot elongating, still soft needles (>3 cm)
6	Shoot fully elongated
7	Needles turning dark green, fully hardened

2.7 Statistical Methods

All analyses were performed in R studio and Microsoft Excel 2010.

2.7.1 Meteorological data

Daily average, minimum and maximum temperature was calculated and plotted for each sensor for the indoor experiment. Mean values of temperature were also calculated within heated and control plots for the outdoor experiment. In this way, the average, the average minimum and the average maximum of temperature of control and heated treatments were obtained and plotted. Wind speed and relative humidity were plotted hour by hour. Millimetres of rain dropped every hour were summed within the day to calculate the daily millimetres of rain. Average, maximum, minimum and standard deviation of soil temperature was calculated for each of the treatment from the soil sensor data.

2.7.2 Temperature curves

For each analysed individual, temperature curves were fitted with a second degree polynomial, doing a squared regression. Temperature optimums (T_{opt}) were calculated as maximums of the functions. Linear mixed-effect models were performed on the temperature curves data to obtain a model of

response for both temperature and light treatments. They explained the assimilation rate as an effect of cuvette temperature, squared of the cuvette temperature, internal PAR of the cuvette, species and temperature treatments [**Eq. (2.1)**]. A model reduction was performed to include only significant interactions.

$$y = T_{cuv}^2 + TempTreat * T_{cuv} + PAR_i * T_{cuv} + Species + \ Error \quad (2.1)$$

Where y is the assimilation rate, T_{cuv} represents the temperatures that were set to perform the temperature curves during the experiment, T_{cuv}^2 are the squared values of T_{cuv}, $TempTreat$ represents the two temperature treatments (*G13* and *G6*), PAR_i is a categorical variable of light levels (100 - 200 µmol m^{-2} s^{-1}) and $Species$ refers to the measured species (*Picea abies* and *Abies alba*) and $Error$ terms are independent and they follow a normal distribution, $N(\mu, \sigma^2)$ that was confirmed by Shapiro-Wilk test ($W = 0.976$, $p = 0.033$). Model assumptions were tested with Normal quantile plot, residual plot and standardized residuals plot. A χ^2 test with the drop-1 function was carried out to understand significance and correlations of variables in the model. The model was chosen considering the lowest AIC.

2.7.3 Light curves

Light curves were fitted by the Solver function of *Microsoft Excel* following Lobo *et al.*, 2013 method, using the nonrectangular hyperbola-based model [**Eq. (2.2)**].

$$P_N = \frac{\phi_{(I_0)} \times I + P_{gmax} - \sqrt{(\phi_{(I_0)} \times I + P_{gmax})^2 - 4\theta \times \phi_{(I_0)} \times I \times P_{gmax}}}{2\theta} - R_D \quad (2.2)$$

Where P_N is the net photosynthetic rate, $\phi_{(I_0)}$ is the quantum yield when I = 0, I is the photosynthetic photon flux density, P_{gmax} is the maximum gross photosynthetic rate, θ is the convexity factor and R_D is the dark respiration. These latter variables were calculated in *Microsoft Excel* using templates developed by Lobo *et al.*, 2013, for all individuals tested. Other parameters were not considered because they were calculated with constants belonging to the machine properties set by the authors, and not relevant for this work. Average values for each light level and treatment were calculated and analysed as function of the temperature treatments [**Eq. (2.3)**].

$$y = TempTreat * Species + Error \quad (2.3)$$

Where y stands for one of the calculated variables (P_{gmax}, $\phi_{(I_0)}$, θ, R_D and Light Compensation Point (*LCP*)), $TempTreat$ is a continuous variable for the temperature treatments, $Species$ is a categorical variable for tested species (*Picea abies* and *Abies alba*) and $Error$ terms are independent and they follow a normal distribution, $N(\mu, \sigma^2)$. Assumptions were tested with normal quantile plot, residual plot and standardized residuals plot. An analysis of covariance was performed using ANOVA function and F test with drop-1 function in R.

2.7.4 Dark Respiration

To analyse respiration in the dark, gas exchange measurements recorded in darkness (PAR < 10 µmol m^{-2} s^{-1}) were considered, more precisely data recorded before sunrise and after sunset during diurnal curves, and ambient measurements in the greenhouses for plants exposed to total dark treatment. Data collected outdoor and indoor were analysed separately. Respiration rate was obtained multiplying assimilation values for -1. Average and standard deviation of respiration were calculated for each treatment. Linear regression models [**Eq.** (**2.4**)] were performed considering respiration a function of temperature recorded in the gas exchange chamber for different species and experimental set up.

$$DR = Tcuv + Error \tag{2.4}$$

Where DR is respirationin in the dark, $Tcuv$ is the temperature recorded in the cuvette during the maesurement, and $Error$ terms are independent and they follow a normal distribution, N(µ, σ^2). Assumptions were tested with normal quantile plot, residual plot and standardized residuals plot. The equations resulted from the models were used to calculate $Q10$ [**Eq.** (**2.5**)].

$$Q10 = \left(\frac{R_2}{R_1}\right)^{\frac{10°C}{(T_2 - T_1)}} \tag{2.5}$$

Where $Q10$ is the *temperature coefficient of respiration*, R_1 and R_2 correspond to the respiration rates calculated respectively for T_1 and T_2, and T_1 and T_2 are temperatures values with $T_2 > T_1$.

A regression model [**Eq.** (**2.6**)], that considered temperature treatment and species as additional variables, was then performed and plotted with a semilogarithmic scale for the respiration values.

$$\log(\mathrm{DR}) = Tcuv * TempTreat + Species + Error \tag{2.6}$$

Where log (DR) is the base 10 logarithm of respiration in the dark, $Tcuv$ is the cuvette temperature, $TempTreat$ is the temperature treatment plants were subjected, $Species$ are tested species (*Abies alba* and *Picea abies*) and $Error$ terms are independent and they follow a normal distribution, N(µ, σ^2). Assumptions of linear models were tested with Normal quantile plot, residual plot and standardized residuals plot. The effect of temperature on respiration was estimated with a Pearson's correlation test.

2.7.5 Diurnal curves

Data collected from the diurnal curves were used to calculate average carbon uptake per individual per species in each treatment. Two linear models, one for each experiment, were performed to show average carbon uptake against temperature and light treatment, and species [**Eq.** (**2.7**)].

$$\mathrm{Cu} = Temp + LightTreat + Species + Error \tag{2.7}$$

Where Cu is the carbon uptake, $Temp$ is the temperature that plants were exposed to, $LightTreat$ is the light treatment, $Species$ are the tested species (*Abies alba* and *Picea abies*), and $Error$ terms are independent and they follow a normal distribution, N(µ, σ^2). Assumptions of linear models were tested with normal quantile plot, residual plot and standardized residuals plot. Effects of variables on the models were tested with ANOVA.

2.7.6 Plant dimension

Diameters and heights collected before the experimental month and at the end of the growing season were used to calculate the growth of each plant subtracting final and initial values. Linear models were built to see if there is a linear effect of temperature and light treatment on growth rate for both height and diameter. Assumptions of linear models were tested with normal quantile plot, residual plot and standardized residuals plot. Box plots were used to evidence differences among the averages for each species.

2.7.7 Phenology

Data collected during the growing season were used to analyse phenology. To compute the data, dates were converted into day number, so that every date corresponds to the number of days elapsed from day number one of measurement. Recorded scores were tested against day number with a non-linear model for each species and temperature treatment first, and for every individual afterwards [**Eq. (2.8)**].

$$Score \sim SSlogis(Dayno, Asym, xmid, Scal) + Error \quad (2.8)$$

Where $Score$ stands for the score given during measurement, $SSlogis$ is the logistic function or sigmoid curve, $Dayno$ is the number of days elapsed from day one to a certain day, $Asym$ (asymptote), $xmid$ (x value corresponding to the inflection point of the curve) and $Scal$ (scale parameter that depends on $Dayno$) are parameters calculated automatically to fit the sigmoid curve on the data. $Error$ terms are independent and they follow a normal distribution, $N(\mu, \sigma^2)$.

Values of $xmid$ were calculated for each individual and compared with the others to investigate the effects of temperature and light treatments on the phenology, in terms of time. An analysis of variance was, therefore, performed on these latter.

3. Results

3.1 Environmental conditions

3.1.1 Temperature

For the outdoor experiment average surface temperatures in the *control* plots were lower than in the *heated* ones (Mean difference = 1.9°C). For the indoor experiment, the average air temperature was the highest in *G13*, followed by *G11*, *G9*, *G6* and *Out*, as we expected [**Tab. 3.1**]. Patterns of temperatures are illustrated in **figure 3.1**. Highest peaks of temperatures were recorded in *G9*, where the maximum overpassed 30°C.

Table 3.1 – Values of temperatures recorded in different treatments. Average (Av.), minimum (Min.) and maximum (Max.) surface (outdoor experiment) and air (indoor experiment) temperatures.

	Temperature (°C)		
Plots	**Av.**	**Min.**	**Max.**
Control outdoor	4.8	-3.3	10.5
Heated outdoor	6.7	-0.9	12.2
G13	13.0	8.6	21.2
G11	10.6	8.1	16.8
G9	8.7	1.0	31.2
G6	5.8	-2.5	16.7
Out	5.2	-3.4	11.2

3.1.2 Light

The highest levels of PAR were reached in *G9* with 7 days with maximums above 2000 μmol m^{-2} s^{-1}, while the lowest outdoor with only one day with a peak above 500 μmol m^{-2} s^{-1}. The average PAR levels and standard deviation recorded during the day light (from 8:00 to 16:00 circa) are presented in **table 3.2**. Patterns of PAR levels during the experimental month are illustrated in [**Fig. 3.2**].

Table 3.2 – Values od PAR recorded in different treatments. PAR average values (Av.) and standard deviation (SD) recorded in the five greenhouses and outside.

	PAR (μmol m^{-2} s^{-1})	
Plots	**Av.**	**SD**
G13	398.06	340.31
G11	382.16	310.16
G9	620.97	705.90
G6	87.05	74.29
Out	135.06	123.64

3.1.3 Rain and wind

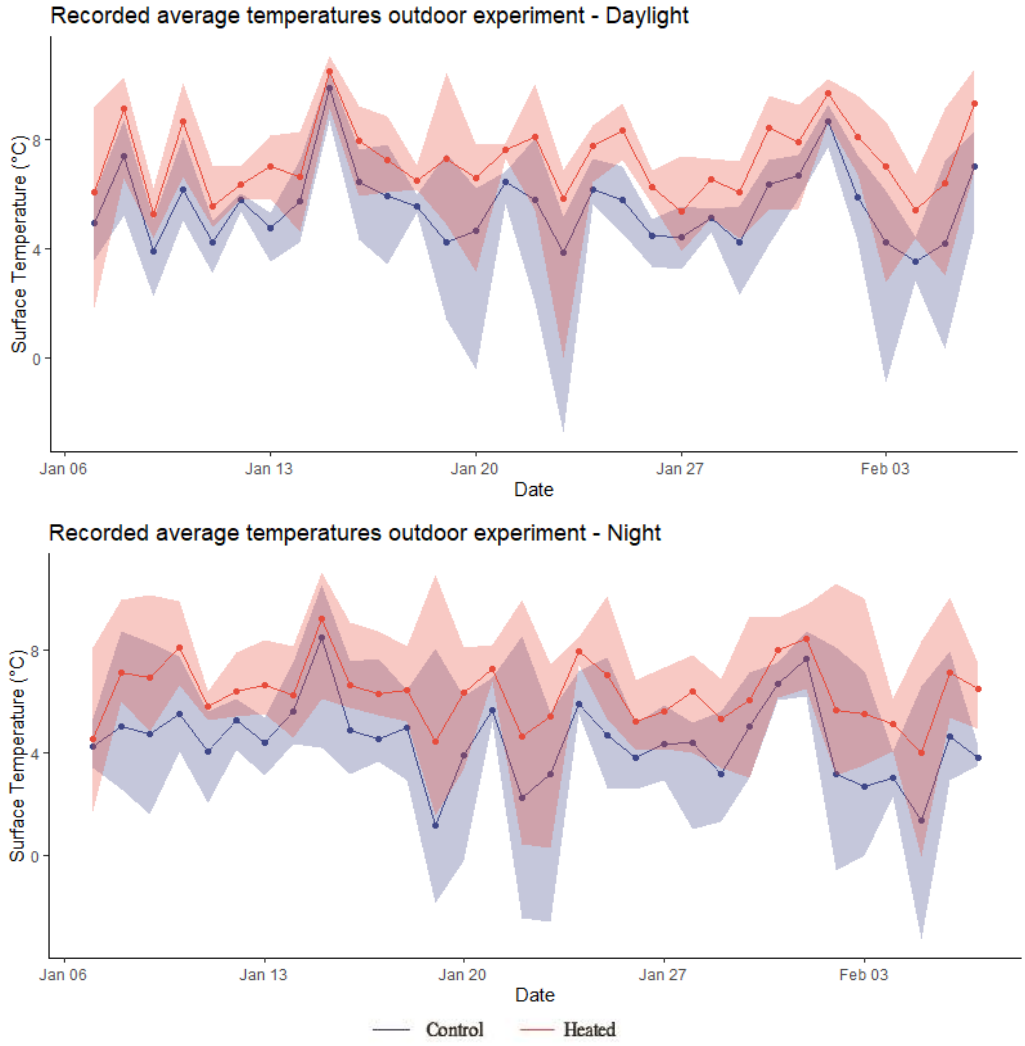
Wind speed and relative humidity recorded are illustrated in **figure 3.3**. Wind speed was influenced by the sensors position inside the canopy, with maximum values of 3 m s^{-1}. Relative humidity was always higher than 75%. Daily rain was calculated, and it results in 17 days of rain (considering a rainy day a day with more than 2.5 mm of rain) during the experimental period [**Fig. 3.4**].

3.1.4 Soil temperature

Table 3.3 presents the average, minimum, maximum and standard deviation of soil temperature in each treatment for the outdoor experiment, whereas **figure 3.5** illustrates soil temperature patterns for *heated*, *control*, *light* and *shade* combination of treatments.

Table 3.3 – **Values of soil temperature recorded in the outdoor experiment.** Average (Av.), minimum (Min.), maximum (Max.) and standard deviation (SD) values of soil temperature.

	Temperature (°C)			
Treatment	**Av.**	**Min.**	**Max.**	**SD**
Heated Light	10.74	3.35	17.27	2.50
Heated Shade	8.47	1.26	1230	1.83
Control Light	5.18	0.32	10.12	1.65
Control Shade	5.05	0.19	10.15	1.71



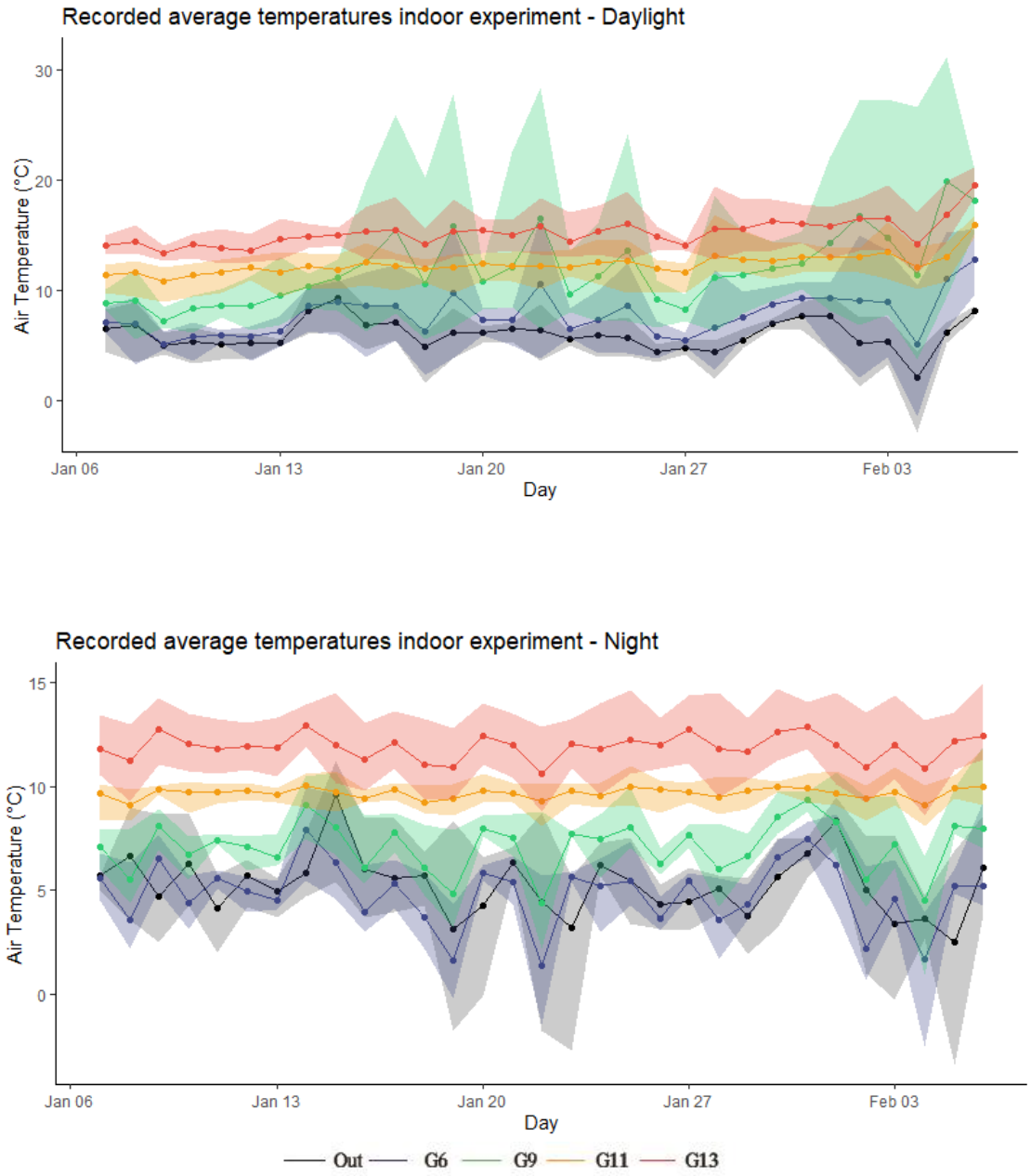


Figure 3.1 – Average, minimum and maximum temperature patterns in the two experiments. First two panels show surface temperature in *control* and *heated* plots, and second two panels evidence air temperature in the four greenhouses and in the control outside. The amplitude of coloured areas evidences the variation of temperature within the same day.

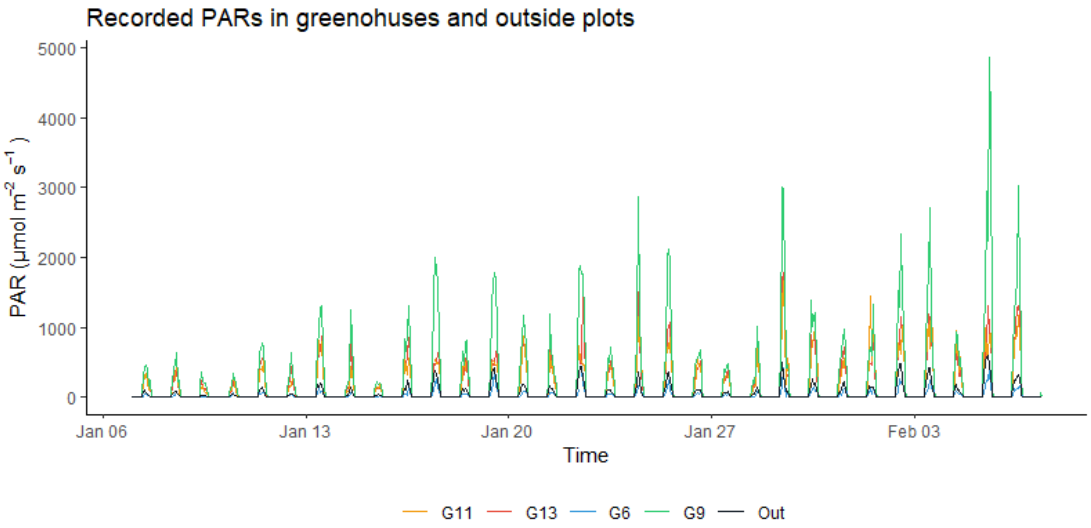


Figure 3.2 – PAR levels. Patterns of PAR levels recorded hour by hour during experimental month from 7th of January to 7th of February in the four greenhouses and outdoor.

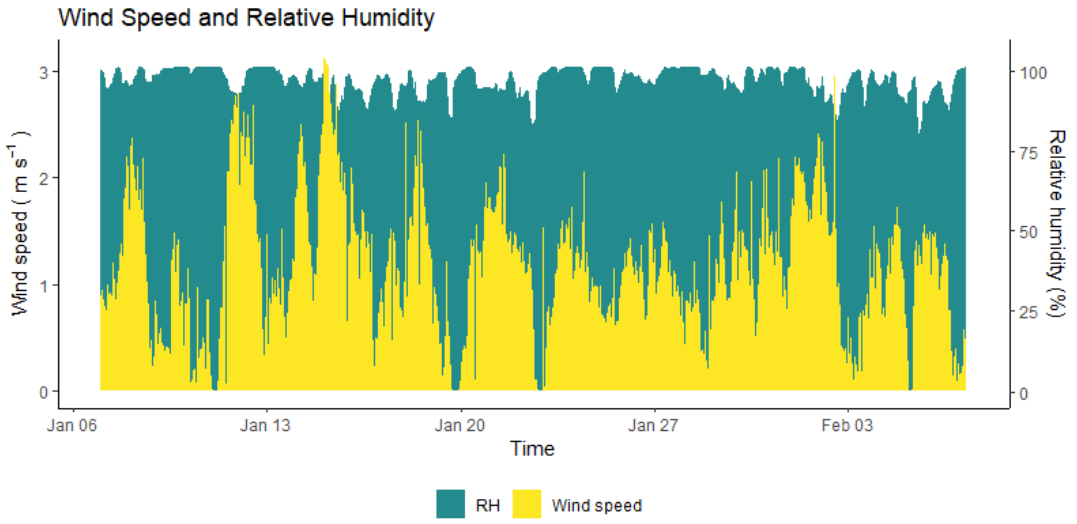


Figure 3.3 – Wind speed and relative humidity. Data are displayed on different scales: left axis shows wind speed (m s^{-1}) and right axis shows RH (%).

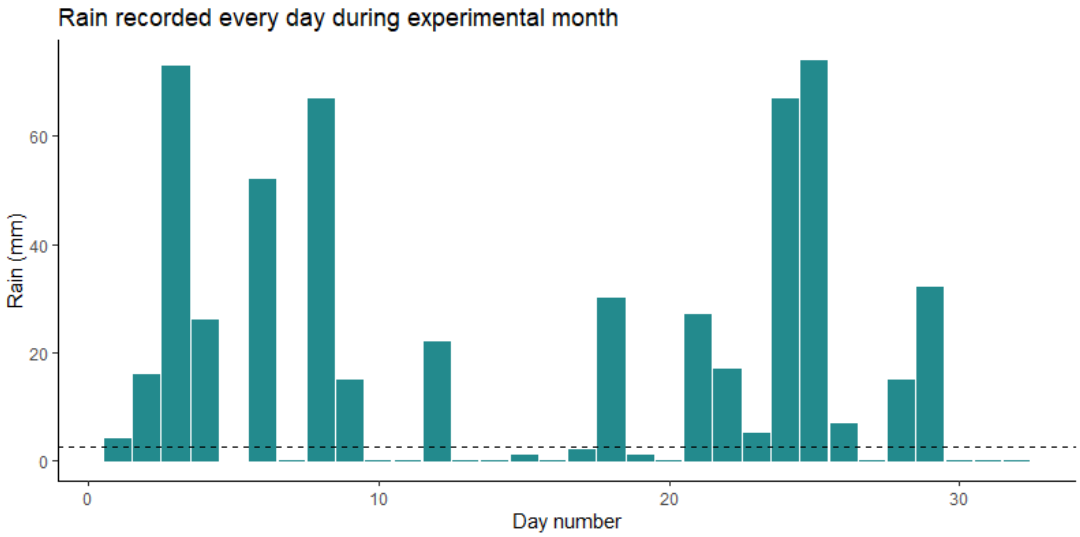


Figure 3.4 – Daily rain recorded during the experimental month. Black dashed line depicts 2.5 mm of rain. Day number 0 corresponds to the beginning of experimental month (7th of January) and day 32nd the end (7th of February).

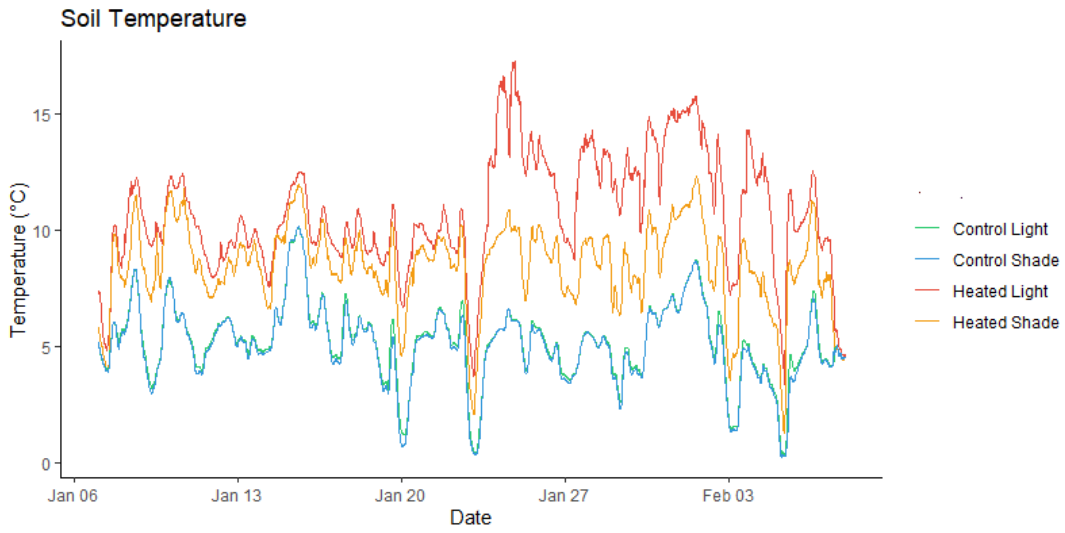


Figure 3.5 – **Soil temperature.** Graph shows the patterns recorded during experimental month.

3.2 Temperature curves

Temperature curves show that the temperature optimum (T_{opt}) varied from a minimum of 3.9° C (recorded at 100 µmol m^{-2} s^{-1} in *G13*) to a maximum of 22.4°C (recorded at 200 µmol m^{-2} s^{-1} in *G6*) [**Annex I**].

Species were not significant in the model ($p > 0.05$), but the other variables show a significant effect on the curves [**Tab. 3.4**]. The curves obtained with the model are illustrated in **figure 3.6.** It appears that curves are affected by temperature treatment and light levels, that shifted the assimilation rate and the T_{opt}. Indeed, assimilation rate was higher for plants subjected to 200 µmol m^{-2} s^{-1}. The T_{opt} appeared to be shifted to the right for plants belonging to *G6*, meaning that plants acclimated to lower temperatures have a higher optimum of temperature.

Table 3.4 – Effect of the variables in the model with Chi-squared (χ^2) test and corresponding p-values. Significance levels are shown in the following way: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Effect	Test (χ^2)	p-value
T_{cuv}^2	25.6354	4.124e-07 ***
$Species$	1.5991	0.206032
$TempTreat * T_{cuv}$	4.6213	0.031577 *
$PAR_i * T_{cuv}$	9.1377	0.002504 **

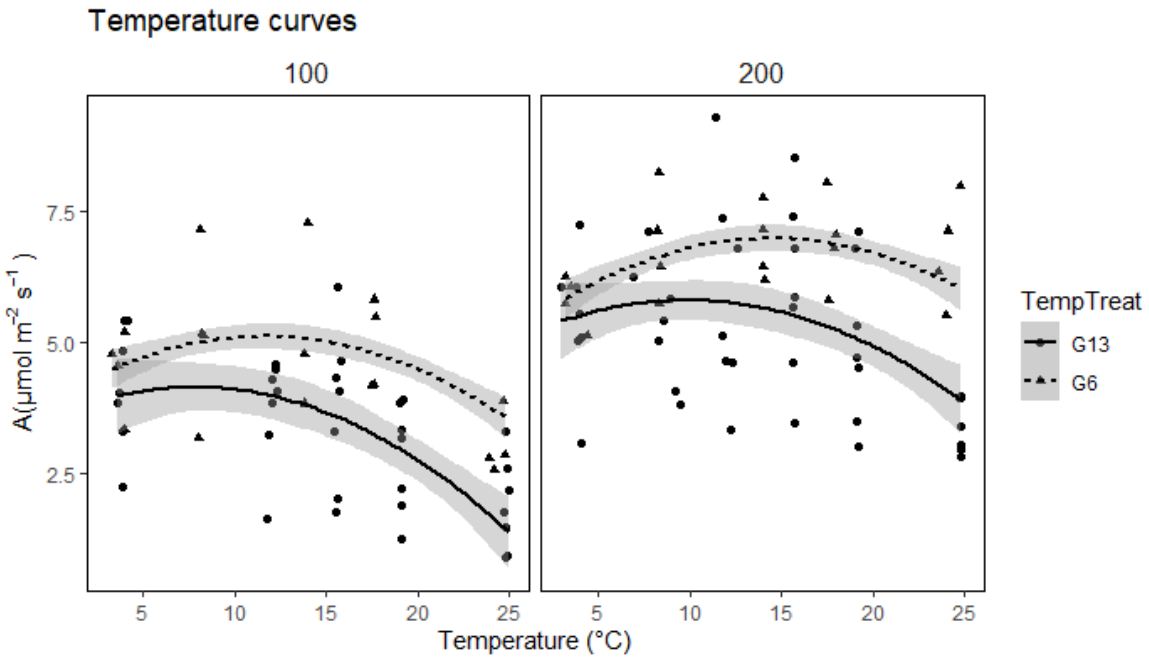


Figure 3.6 – **Temperature curves fitted with linear mixed-effect model.** Light exposition used to perform the curves was 100 µmol m^{-2} s^{-1} in the left panel, and 200 µmol m^{-2} s^{-1} in the right one. *G13* and *G6* are the highest and lowest temperature treatment used in the indoor experiment. Conifers in *G13* show lower assimilation than the ones in *G6*. The graphs also evidence how the T_{opt} (*x* axis value corresponding to the maximum of the curve) is slightly higher for plants grown in *G6*.

3.3 Light curves

All the Light curves were plotted [**Annex II**] Average of calculated variables are reported in the **table 3.5**, while all the calculated parameters are shown in the **annex II**. The covariance analysis showed that P_{gmax} differed between species and that temperature treatment had an effect on R_D [**Tab. 3.6]**. The other variables did not show any significant effect of species, temperature treatment and interaction between the two of them. Regression lines were plotted [**Fig. 3.7**], only P_{gmax} and R_D are shown below, the others are displayed in the **annex II.**

Table 3.5 – **Average values of estimated variables from light curves.** Values are divided by different temperature treatments and species.

Plot	Species	$\Phi_{(I_0)}$ (µmol mmol^{-1})	P_{gmax} (µmol m^{-2} s^{-1})	θ	R_D (µmol m^{-2} s^{-1})	LCP (µmol m^{-2} s^{-1})	Temperature Treatment (°C)
G13	*Picea abies*	0.077	9.421	0.937	1.029	13.578	12.985
G13	*Abies alba*	0.089	12.170	0.868	1.349	17.269	12.985
G11	*Picea abies*	0.052	8.056	0.924	0.884	19.038	10.549
G11	*Abies alba*	0.057	11.481	0.932	0.756	14.175	10.549
G9	*Picea abies*	0.047	9.037	0.934	0.722	15.568	8.660
G9	*Abies alba*	0.101	13.685	0.657	1.044	13.650	8.660
G6	*Picea abies*	0.064	8.076	0.751	0.662	10.559	5.766
G6	*Abies alba*	0.106	12.199	0.800	0.542	5.758	5.766

Table 3.6 – Effect of temperature treatment on light curves parameters. F values and p-values of Temperature treatment species and interaction of the two of them for P_{gmax} and R_D. Significance levels are evidenced in this way: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	P_{gmax}		R_D	
Effect	**F value**	**p-value**	**F value**	**p-value**
TempTreat	0.069	0.810	9.556	0.037*
Species	33.668	0.004**	0.615	0.477
TempTreat*:*Species	0.771	0.429	0.770	0.430

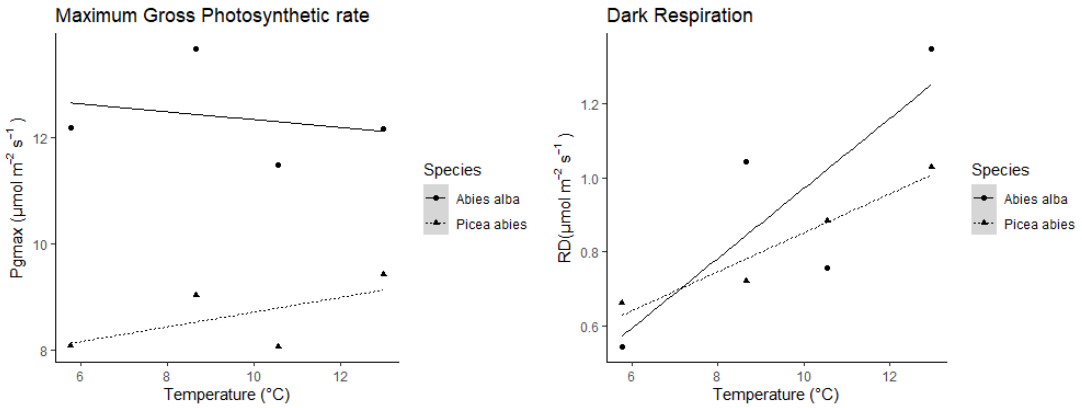


Figure 3.7 – **Temperature treatment effect on gross photosynthetic rate and dark respiration.** Regression lines of average values of maximum gross photosynthetic rate (P_{gmax}) and dark respiration (R_D) plotted as function of temperature treatment, for *Abies alba* (continuous line) and *Picea abies* (dashed line).

3.4 Dark Respiration

Average values of dark respiration and relative standard deviation are shown in **figure 3.8.** Looking at the temperature treatments, it appears that average respiration is higher in warmer greenhouses. The obtained equations and *Temperature coefficients of respiration* (*Q10*) are shown in the **table 3.7**.

The regression analysis shows that the cuvette temperature has a positive effect on in the indoor experiment respiration (cor = 0.549, p = <0.05), while it has no effect in the outdoor one (cor = 0.035, p > 0.05). Regressions lines were plotted for both the experiments [**Fig. 3.9**] obtaining different effects of the variables on the model [**Tab. 3.8**].

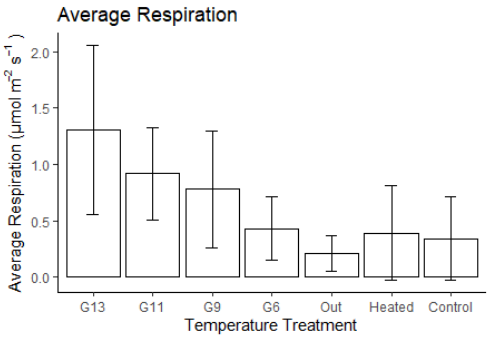
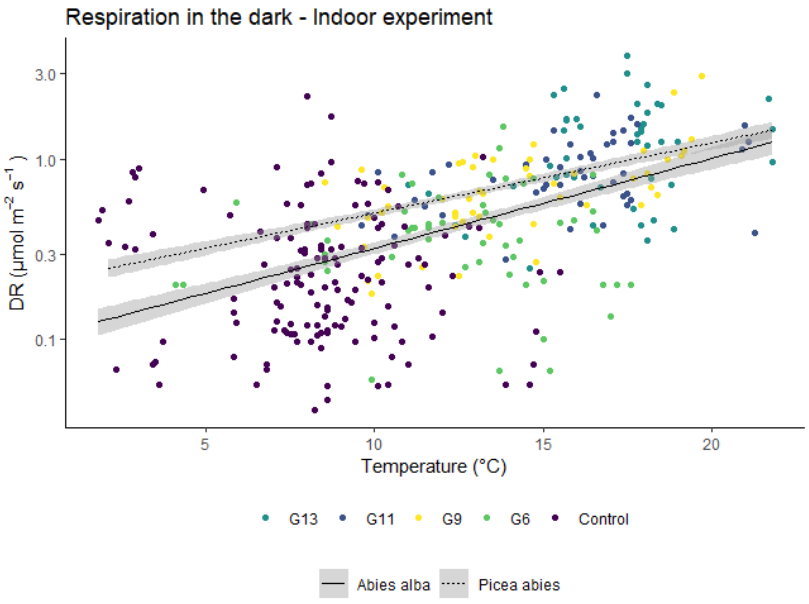


Figure 3.8 – **Average respiration.** Mean dark respiration values (Av.R) and standard deviation (SD) in the different temperature treatments for both experiments.

Table 3.7 – Dark respiration as function of cuvette temperature. Lines equations obtained from the regression analysis and *Q10* calculated with the **eq. 3.5**.

	Equations	**Q10**
***Abies alba* - outdoor**	$DR = 0.281$	0.88
***Picea abies* - outdoor**	$DR = 0.02\,Tcuv + 0.332$	1.33
***Abies alba* - indoor**	$DR = 0.06\,Tcuv - 0.164$	2.41
***Picea abies* - indoor**	$DR = 0.08\,Tcuv - 0.266$	2.50



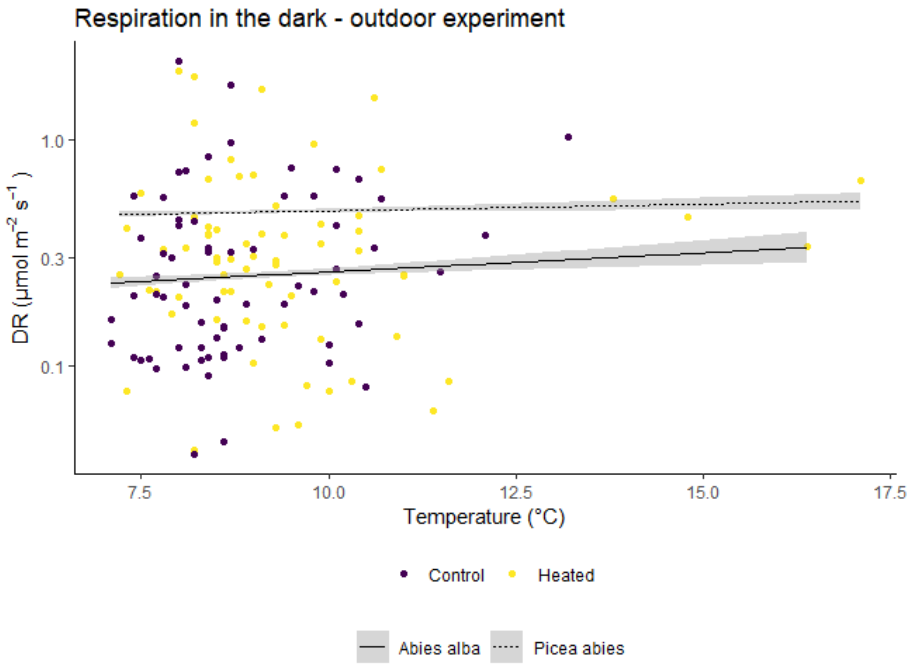


Figure 3.9 – **Linear regression models of dark respiration.** Dark respiration (DR) models were calculated with [**Eq.** (**3.6**)]. First panel shows the model obtained from indoor experiment, and second panel shows the model obtained from outdoor experiment. Regression for *Abies alba* and *Picea abies* are respectively plotted with continuous and dashed lines.

Table 3.8 – **Effect of model variables on dark respiration.** F values and p-values obtained by ANOVA on the linear models performed with [**Eq.** (**3.6**)] for both indoor and outdoor experiments. Significance levels are shown in the following way: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Indoor experiment		**Outdoor experiment**	
Effect	**F value**	**p-value**	**F value**	**p-value**
Tcuv	208.9123	< 2.2e-16 ***	0.1858	0.6670738
TempTreat	21.7278	5.203e-16 ***	0.4317	0.5122495
Species	17.6256	3.424e-05 ***	12.6644	0.0005116 ***
Tcuv*:*TempTreat	8.4028	1.767e-06 ***	0.4970	0.4820229

3.5 Diurnal Curves

Average carbon uptake for each species and treatment was calculated [**Fig. 3.10**]. The model for the outdoor experiment showed no effect of temperature and species ($p > 0.05$) on the average carbon uptake but revealed significant effects of light treatment ($p < 0.05$). Temperature and light treatments were both significant ($p < 0.05$) in the indoor experiment, on the other hand species did not show any significant difference ($p > 0.05$) between each other. Regression lines are shown in **figure 3.11**, evincing the negative effect of temperature on carbon uptake in the indoor experiment.

3.6 Plant dimension

3.6.1 Indoor experiment

Temperature treatment had a negative effect on *Larix X eurolepis* growth in height ($p < 0.05$), but not in diameter. It shows instead a positive effect on *Fagus sylvatica* growth in height ($p < 0.05$) but not in diameter. There was not significant effect of temperature on the growth in height and in diameter for

both *Abies alba* and *Picea abies*. Light treatment, instead, shows a positive effect on *Quercus robur* growth in diameter ($p < 0.05$) but not in height. It doesn't show any effect on the other species. **Figure 3.12** reassumes effect of light and temperature treatments on the growth for indoor experiments (plots where there was not any significance are shown in **annex III**).

3.6.2 Outdoor experiment

None of the species show any effect of temperature on growth in height and dimeter. Light shows a negative effect on *Quercus robur* growth in diameter ($p < 0.05$), but not in height. Light does not have any effect on growth of the other species. **Figure 3.13** reassumes the significant effect of light and temperature treatments on the growth for both indoor experiments (plots where there was not any significance are shown in **annex III**).

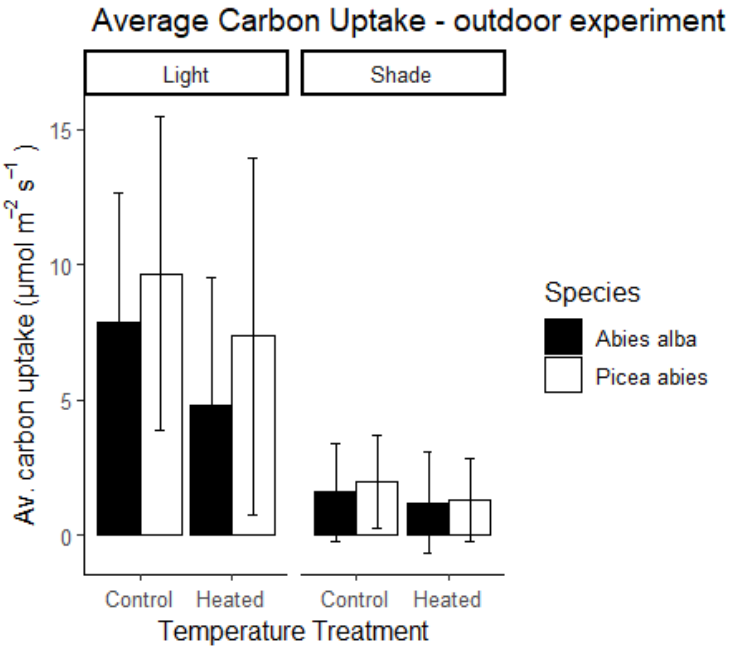
3.7 Phenology

3.7.1 Indoor experiment

Non-linear regression analysis showed that sigmoid curves fitted data significantly ($p < 0.05$) [**Fig. 3.14**]. The day numbers corresponding to the inflection point of sigmoid functions performed for each individual are represented in the boxplot [**Fig. 3.15**]. From the statistical analysis of the inflection points, it appears that temperature treatment had a significative positive effect on the phenology for *Quercus robur* ($p < 0.05$), but not for the other species ($p > 0.05$). In contrast, light treatment showed positive effect on both *Picea abies* ($p < 0.05$) and *Quercus robur* ($p < 0.05$).

3.7.2 Outdoor experiment

Non-linear regression analysis showed that sigmoid curves did not fit the data significantly ($p > 0.05$) [**Fig. 3.16**]. Further analyses were, therefore, not possible to accomplish.



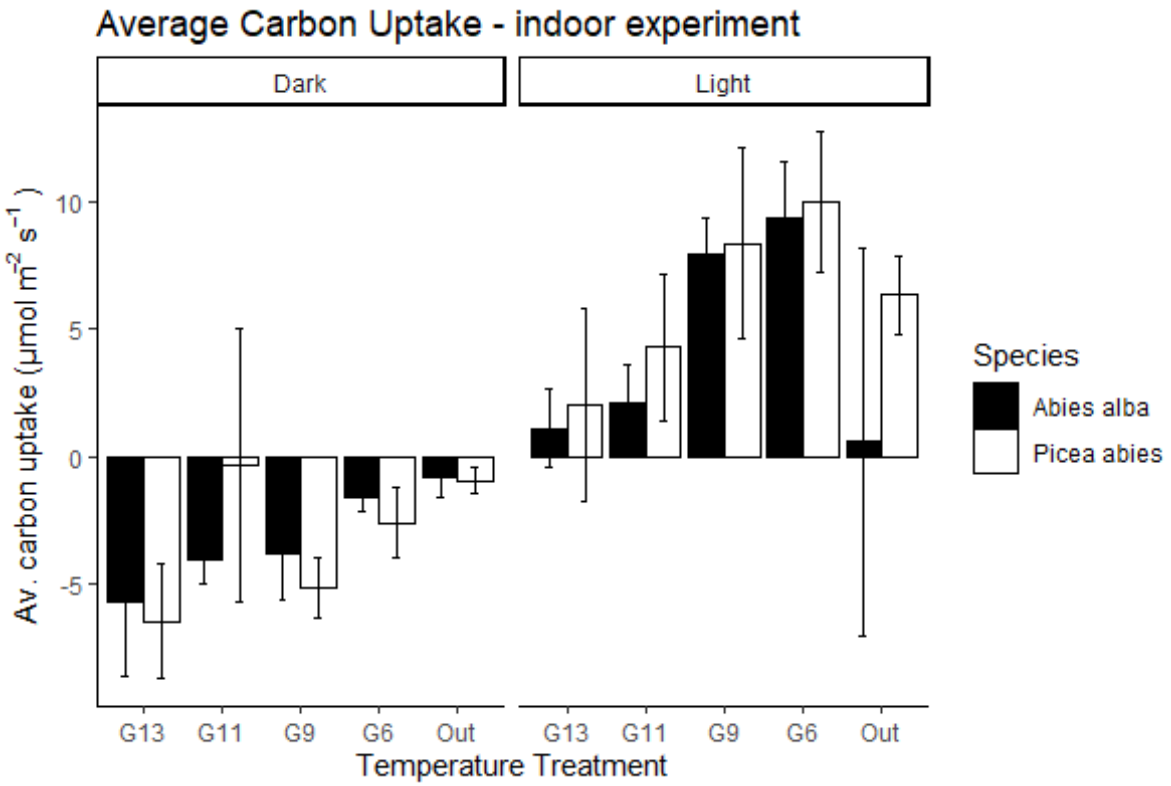
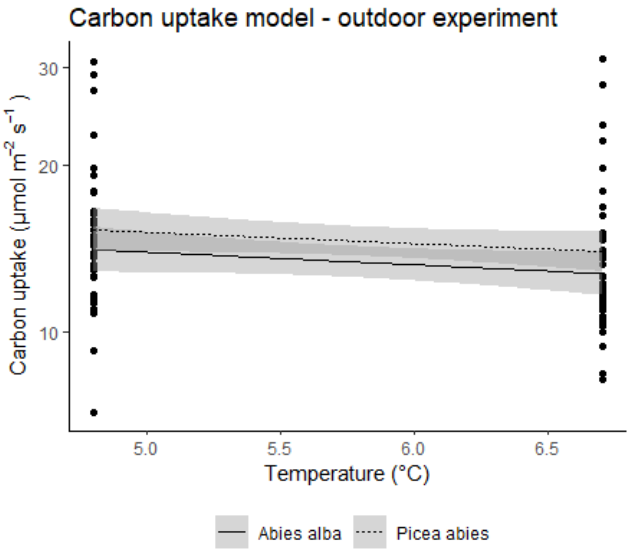


Figure 3.10 – Average carbon uptake in the two experiments. Graphs distinguish temperature treatments, light treatments and species.



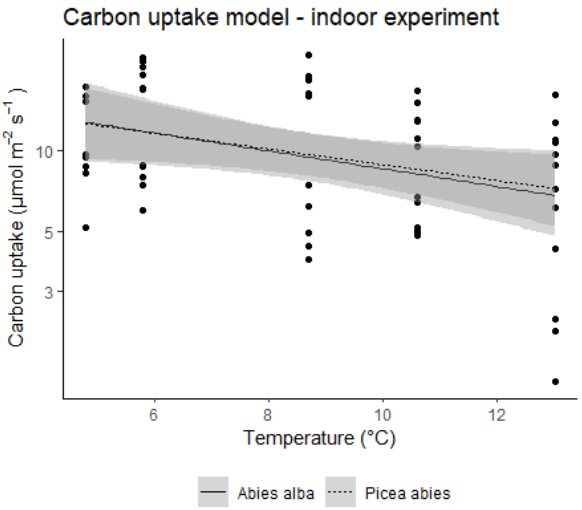


Figure 3.11 – Carbon uptake model for the two experiments. Carbon uptake values were transformed with a base 10 logarithm, after being increased of ten units. Temperature effect is negligible in the outdoor experiment, but it shows a negative effect on the carbon uptake in the indoor one.

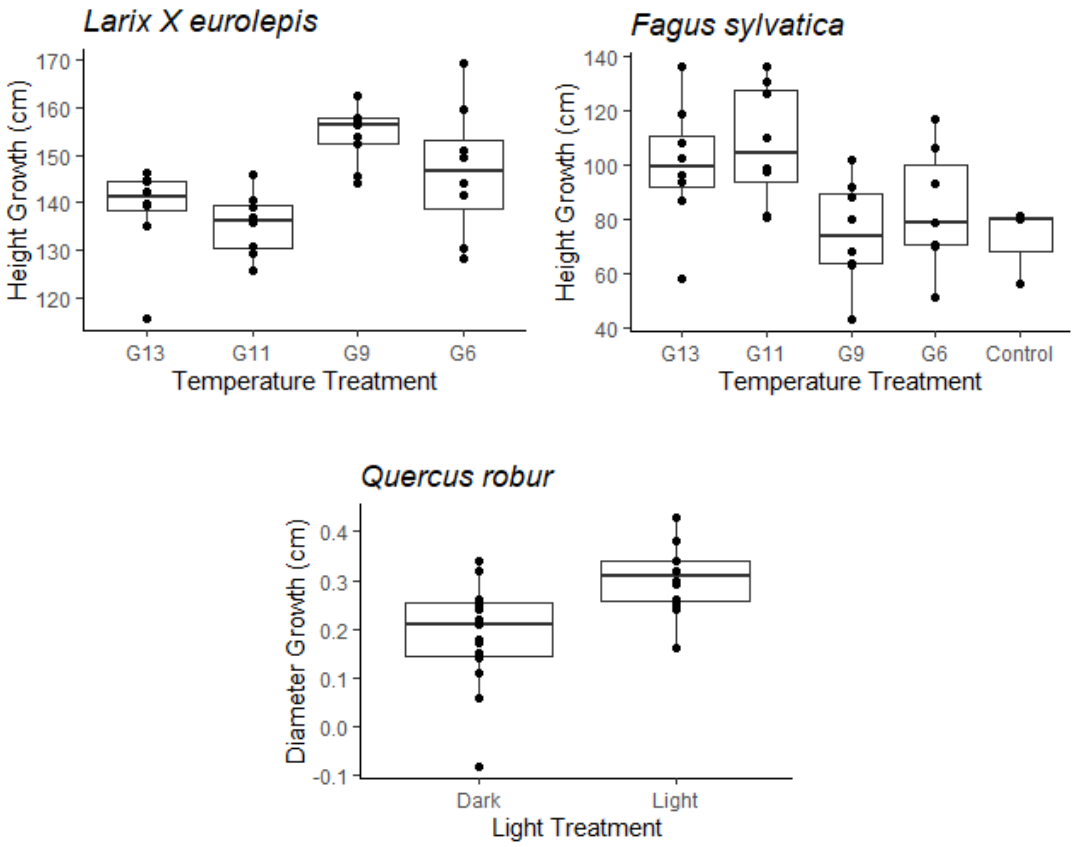


Figure 3.12 – Temperature and light treatment effect on the growth – indoor experiment. Different species showed different behaviours on growth due to light and temperature treatment.

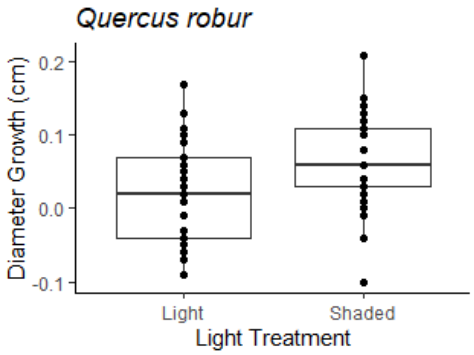
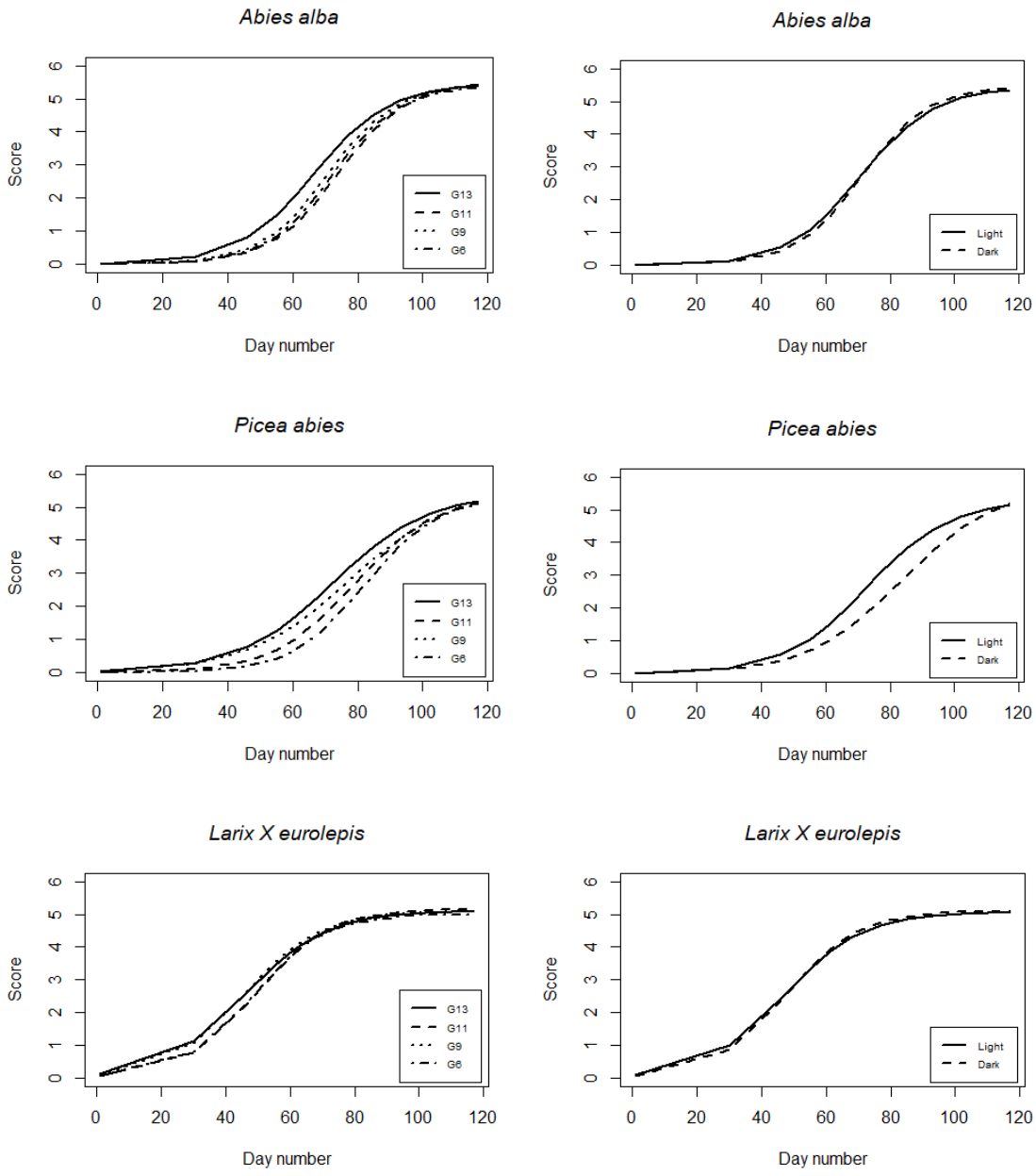


Figure 3.13 – **Temperature and light treatment effect on the growth – outdoor experiment.** Different species showed different behaviours on growth due to light and temperature treatment.



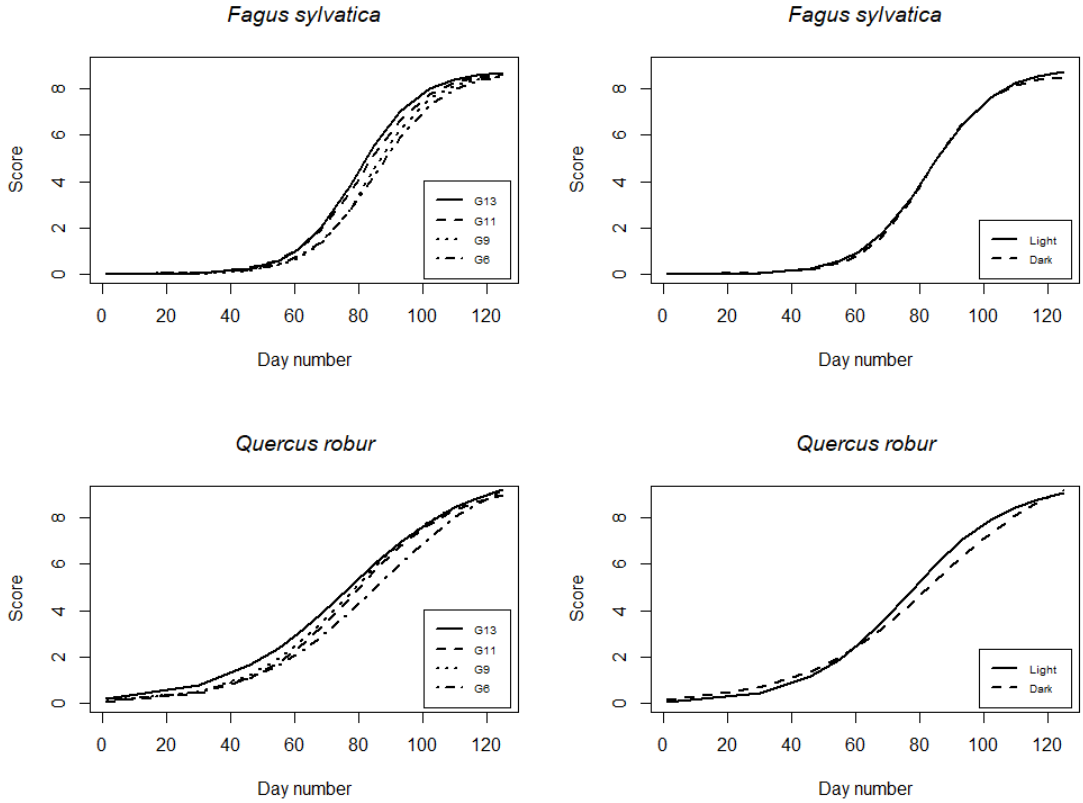
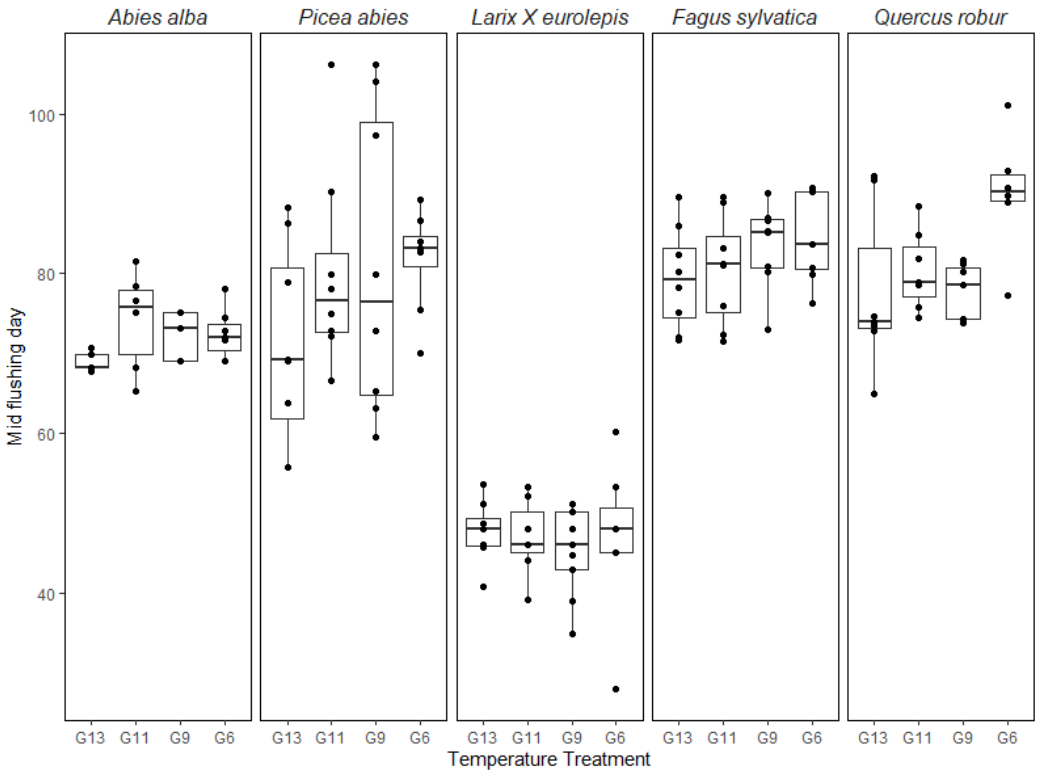


Figure 3.14 – Effect of day number on phenology – indoor experiment. Sigmoid curves explain the effect of day number on the score for the five analysed species. First five panels distinguish for temperature treatment. In these latter, it is evincible that the earliest blooming happened for plants belonging to *G13*. The second six panels differentiate, instead, for light treatment. Lines overlapped in all the species beside *Picea abies*, in which plants exposed to light showed an earlier blooming.



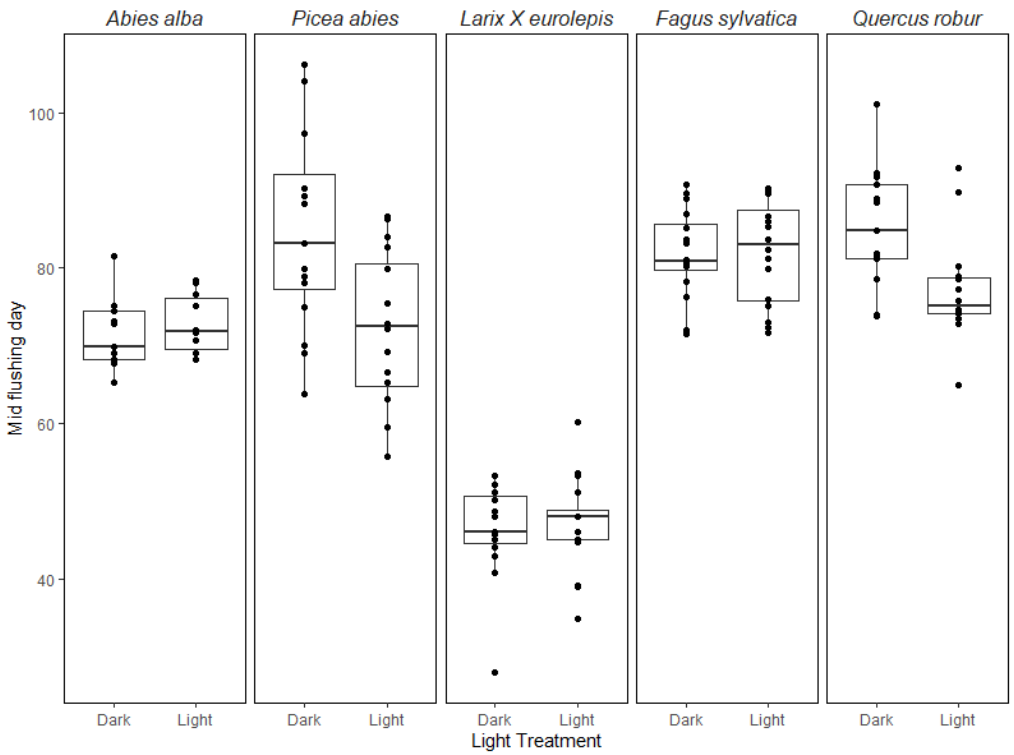
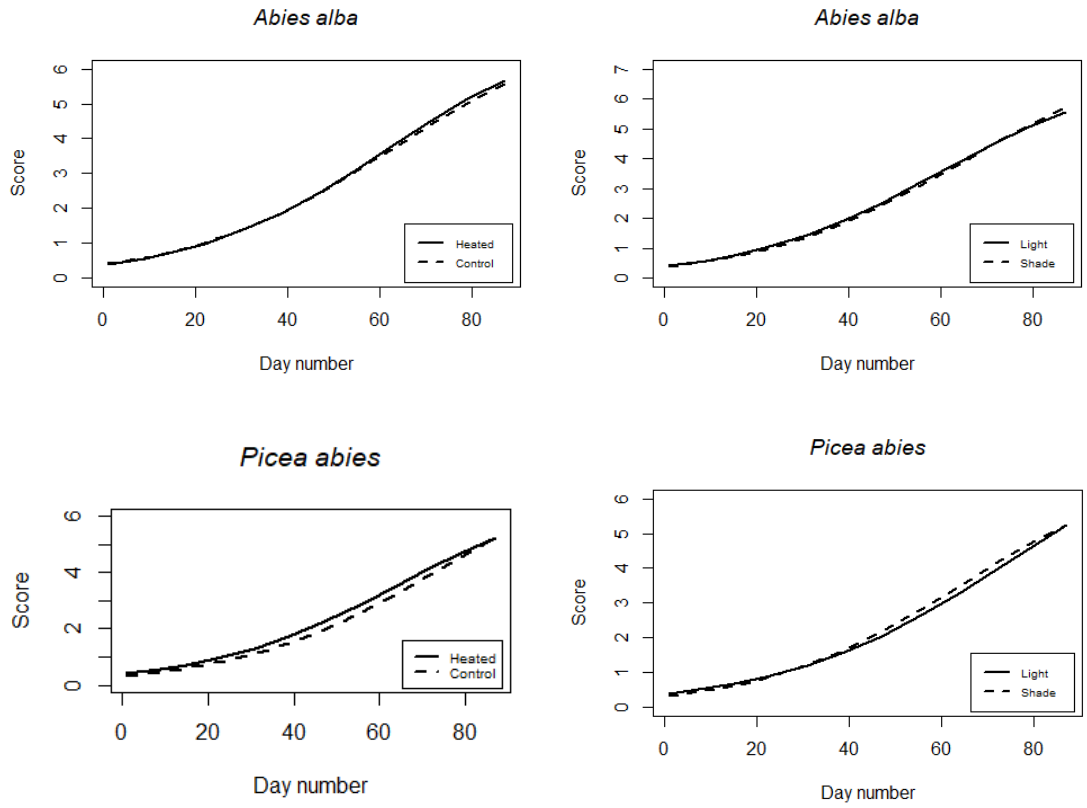


Figure 3.15 – **Mid flushing point related to temperature and light treatment.** Mid flushing day represents the inflection point calculated from the sigmoid curves performed on individuals. A lower mid flushing day represents an earlier flushing of the plant during the growing season. First panel shows mid flushing day related to temperature treatment, whereas the second relates md flushing day to light treatment. Temperature treatment had a significant effect on *Quercus robur* and light treatment had a significant effect on *Picea abies* and *Quercus robur*.



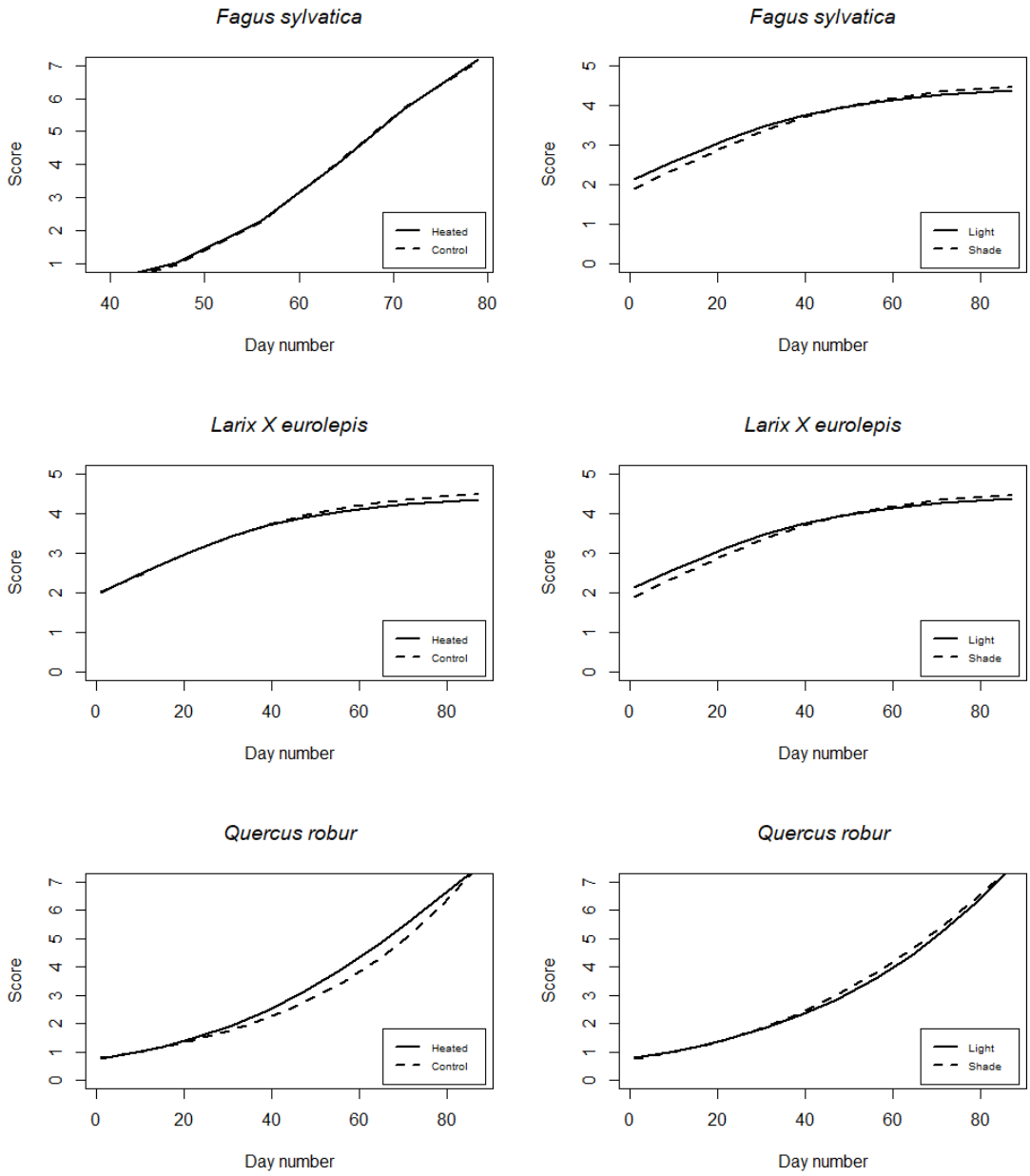


Figure 3.16 – Effect of day number on phenology – outdoor experiment. Sigmoid curves explain the effect of day number on the score for the five analysed species for outdoor experiment. Left panels differentiate for temperature treatment, instead, right panels show differences in light treatment.

4. Discussion

4.1 Environmental conditions

The mean temperature recorded nationwide in Denmark during the month of January was 5.5°C. It was the warmest January since 1874. The average temperature recorded in the last 30 years during the same month was 1.6°C (Rubek, 2020). It means that the *control* plots already experienced a very warm condition.

The recorded average in the *heated* plot was only 1.9°C above the control, so it was not possible to reach 4°C more as we wanted. This issue seemed to be linked with the position of the thermometer and seedlings, that were not enough protected by wind. However, in the outside set-up, the *heated* plots experienced a higher temperature than the *control* ones during the whole experimental month. There were only three days in which plants experienced night frost in *control* plots, whereas temperature didn't reach negative temperature in the *heated* ones. Temperature excursion was slightly higher during the night than during the day.

In the indoor experiment, mean temperature was in accord with what was expected to be measured. Mean temperature sequence was, indeed, respecting the hierarchy of highest temperature in *G13* and lowest in *G6*, followed, of course by control outside. *G9* showed some high and extreme values during the day, reaching six times a maximum temperature higher than 20°C. This problem didn't occur during the night. *Control* plot and *G6* experienced three nights with a minimum temperature below zero.

According to our data, the intensity of light recorded in the greenhouses was slightly higher than in the control outside. A problem occurred in *G9* where PAR levels overpassed 2000 μmol m^{-2} s^{-1} seven times. These values are not reliable with the average amount of light recorded in Danish winters. It means that a problem with the PAR sensor could have occurred. On the other hand, *G6* showed the lowest levels of PAR, meaning that there was less light in average than outside, it was probably due to the position of the greenhouse. Since PAR data were not totally reliable, they were not used to model plants performances. Nevertheless, recorded hours of sun during January in Denmark where 36.1. They are the 30% less than the average recorded in the last 30 years (Rubek, 2020). So, we could say that the light levels are explicative of a future climate change scenario, where we expect warmer, greyer winters (Chirstensen, 2018).

Nationwide the amount of rain fell in January was 76.9 millimetres, that is 18% more than the average of the last 30 years (Rubek, 2020). The latter was lower than what was recorded by the metereological station we reffered to.

Soil sensors show a sudden change after two weeks of data, probably linked to the moving. The sensors were, indeed, moved at the half of the experiment and since then the data do not show reliable patterns. The temperatures of *heated light* and *heated shade* treatments are too different from each other in the second half of the experiment, whereas the *control light* and *control shade* did not show such a difference in the same period. This means that probably soil sensors were not placed correctly in the port ground. In addition, *heated light* sensors recorded temperatures above 15 °C, which are very not likely to had been with a maximum control temperature of 8°C.

4.2 Plant performances: temperature and light curves

4.2.1 Temperature curves

The performances of both group of plants were lower in the curves performed at a PAR level of 100 μmol m^{-2} s^{-1}. Both the assimilation rates increased, indeed, under 200 μmol m^{-2} s^{-1} PAR. Therefore, light was a limiting factor for assimilation, in the first scenario (Perchorowicz *et al*., 1981; Dietz and Heber, 1986). Very low levels of light limit the assimilation rate of the conifers. Nonetheless, the T_{opt} also changed from the two light levels scenarios. In the curves performed with more light optimums of temperature increased of ≈3°C, for all the plants set respectively in *G6* and *G13*. The amount of light did not allow the plants to perform at their maximum rates. The choice to perform temperature curves at such low PAR resemble the typical amount of light that plants could receive during Danish winters. It is interesting how a very low PAR limits significantly the assimilation rate. Hence, we must consider the future weather conditions (increasing amount of clouds) to model photosynthetic data.

Looking at the temperature treatments, it appears that plants acclimated to lower temperatures had the best performance. Hence, the assimilation rate was higher for those belonging to *G6.* Since the species were not significantly different from each other, it means that both *Picea abies* and *Abies alba* perform better if exposed to lower temperatures. It seems, indeed, that they didn't really acclimate to such a warm condition (13°C) in *G13*. They could show a *detractive* adjustment behaviour, namely a reduction of assimilation rate if the plants are exposed to warmer temperature. Not all the species are, in fact, able to acclimate to warmer temperatures in the same way. Evergreen trees do not very likely adjust their performances to the growth temperature (Wan and Yamori, 2013). The assimilation rate could also be limited by the amount of photorespiration. Since dark and light respiration increases with temperature, seedlings that grow at a higher temperature have a higher respiration rate that results in a lower net assimilation rate (Wan and Yamori, 2013).

T_{opt} also differs between the two temperature treatments at both light levels. The T_{opt} of *G13* plants was ≈5°C lower than T_{opt} of *G6* plants. The same difference is conserved in the 200 μmol m^{-2} s^{-1} PAR temperature curves. Meaning that conifers that grew at lower temperatures show a higher T_{opt}. This look like a contradiction, but if we analysed the matter in the same context, we analysed previous parameters, it is likely to observe this behaviour. On one hand, a low acclimation of plants to higher temperature, diminished the performances of the latter under warmer conditions. So, seedlings were not able to modify their performances to enhance assimilation under different temperature conditions (Wan and Yamori, 2013). On the other hand, respiration rate increased with temperature lowering the performance of the plants (Atkin and Tjoelker, 2003, Wan and Yamori, 2013).

These consequences support the idea that photorespiration acclimates faster to temperature than assimilation does (Wan and Yamori, 2013). This is can be linked with the carboxylation and oxygenation rate of Rubisco. If we consider that the carboxylation rate increases faster than the oxygenation at increasing temperatures (Farquhar *et al*., 1980), it is likely to observe an increasing of respiration at higher growth temperatures and not a rise of photosynthetic rate. Therefore, the risk of respiration increasing quicker than fixation could lead to a negative carbon balance at warmer temperatures.

4.2.2 Light curves

Most of the calculated parameters did not show any difference among species or temperature treatment. Nevertheless, P_{gmax} and R_D show interesting patterns. First, P_{gmax} was different between the two species. *Abies alba* had a higher *Pgmax* than *Picea abies*, in all the temperature treatment. This means that the photosynthetic rate at the light saturation point is higher in *Abies alba*. Differences of P_{gmax} are strictly related with the plant ability to acclimate to different environmental conditions (Jurik *et al*., 1988; Dreyer *et al*., 2001). Nevertheless, there was not significant change of the parameter with temperature treatment. The maximum photosynthetic capacity of the plants could also be related with species-specific stomata conductance and concentration of abscisic acid (Aasamaa *et al*., 2002).

On the other hand, R_D does not show any difference between the species. The patterns of the lines are, indeed, only slightly different from each other. Whereas there is a clear effect of temperature on both. The average respiration rate calculated in each of the treatment, is higher in the higher temperature treatment. Meaning that the R_D is positively related with the growth temperatures (e.g. Atkin and Tjoelker, 2003, Wan and Yamori, 2013).

4.3 Respiration and carbon uptake

4.3.1 Dark respiration is influenced by temperature and temperature treatment

Average respiration measured in the dark was higher in temperature treatments with warmer temperatures in both the indoor and outdoor experiment. The lowest mean value recorded in the greenhouses was one third of the maximum, that was found in *G13*. Meaning that a variation of 7°C increases of three times the respiration rate. The difference among the outside plots was not that relevant as the one found in the greenhouses. But still, a higher mean value of respiration was found in the *heated* plots. Although the standard variation was big enough to reverse the results. However, 1.9°C of temperature more leaded to 15% higher level of respiration. The conditions of the two experiments were not comparable, so it was not possible to build a model that covered both the very low temperatures and the highest recorded in the greenhouses.

The equations calculated by the simple model of dark respiration as a function of temperature measured in the cuvette, without considering the effect of temperature treatments, show the same pattern of the average values. Namely, they have very small values of slope, meaning that temperature did not have a significant effect on this experimental set-up. On the other hand, the equations of indoor experiment show a significant slope revealing an effect of temperature on R_D. The equations looked also different for the two species. *Picea abies* shows indeed a very small slope, whether *Abies alba* has zero slope in the outdoor experiment. *Picea abies* has a higher slope than *Abies alba* in the indoor experiment too, showing the same trend of indoor experiment. It means that in both the experiments, *Picea abies* has a faster increasing of respiration with temperature than *Abies alba*. The *Q10* calculated from the obtained equations follow, of course, the same trend. *Q10* of *Picea abies* in the outdoor experiment is 51% higher than *Abies alba*. Nevertheless, the difference of *Q10* between the two species is not that marked in the indoor experiment. *Picea abies* shows a *Q10* that is only 3% higher than *Abies alba*. *Q10* seems to increase with temperature, contrarily to what is supported in many studies (e.g. Atkin and Tjoelker, 2003). More precise measurements are needed to clarify our results.

The models obtained from the two experiments show different trends for the indoor and outdoor experiment. In the first one there is a significant correlation of dark respiration with the temperature, where once again *Picea abies* has a higher intercept than *Abies alba*, but the latter shows a slightly higher slope. Regression lines resulted from the outdoor experiment do not show any correlation with temperature, but a significance difference between the species. The intercept of *Picea abies* is, once again higher than *Abies alba*. It means that the respiration in the darkness of the first one is higher than the latter. The reason why there is a strong correlation with temperature in the indoor experiment and no correlation in the second is the lack of measurements at higher temperatures in the second one. The difference between the two treatments is, in fact, very small, only 1.9°C, and most of the measurements are gathered in the interval 5-10°C. Whereas in the indoor experiment the range of measurements is wider, from 5°C to more than 20°, with many differences between the temperature treatments.

We can anyway conclude that dark respiration exponentially increases with the temperature recorded at the moment of the measurement but also with the growth temperature and with the interaction of both. Meaning that seedlings which grow under warmer temperatures could show a higher respiration rate than the ones that grow under cooler temperatures (Tjoelker *et al*., 1999; Atkin and Tjoelker, 2003; King *et al*., 2006; Kurepin *et al*., 2018).

4.3.2 The effect of temperature on carbon uptake

Carbon uptake calculated from the diurnal curves show an interesting pattern in both the experiments. In the outdoor experiment, we can suddenly see how the difference is marked between the light treatments. Seedlings that were subjected to shade treatment have a lower average net assimilation. If the average carbon uptake was between 5 μmol m^{-2} s^{-1} and 10 μmol m^{-2} s^{-1} in the ambient light treatment, it was under 5 μmol m^{-2} s^{-1} in the shade one. This means that the 60% less of light could halve the diurnal carbon uptake. These results are interesting if considering the climate change scenario in experimental area. Winters will become greyer than before, due to an increasing of cloud cover (Chirstensen, 2018). A lower amount of light that plants will receive could lead to a slower carbon uptake in cold months, following what we found in this experiment. On the other hand, this question should be better analysed, because many studies show that the radiation that spread through the cloud cover will increase the amount of light received by the plants, resulting in higher assimilation rate (Young and Smith, 1983; Doughty *et al*., 2010). Nevertheless, other studies show a decrease in carbon uptake under cloud cover conditions (Graham *et al*., 2003; Dahl *et al*., 2017). Discrepancies are linked to the amount of light that is diffused or retained by clouds. However, it is important to estimate whether one or the other effect prevail.

There is a difference in average carbon uptake among temperature treatments in both the experiments. In the outdoor experiment, plants subjected to ambient light show a larger difference between the treatments than the ones under shaded conditions. The controls have indeed between 30-50% higher average of carbon uptake than heated plots in the *ambient light* treatment, and an irrelevant difference in the *shade* one. However, heated plants show a smaller carbon uptake than the controls. This difference is much more evident in the indoor experiment. In this case, the carbon uptake recorded in *G6* is four times higher than in *G13*. Recording the biggest jump between *G9* and *G11*. This means that there could be a negative exponential relation between carbon uptake and temperature. Despite this we expected that temperature increases the photosynthetic activity, thus the carbon uptake under warmer conditions (Delpierre *et al*., 2009). But this can be reversed considering evergreen plants during mild winters, were photosynthesis is thought to be more negligible (Printz,

1933). In this scenario, the carbon uptake reveals a negative correlation with temperature. In addition, it has been seen by Piao *et al*., (2008) that an increased carbon uptake happens during growing season, meaning spring months, and an overall carbon loss under mild autumnal conditions. Hence, temperature and length of autumnal season could also lead to a carbon loss (Barichivich *et al*., 2013). Considering this, the balance could get worse if we include a very low carbon uptake during winters due temperature rise. Therefore, annual carbon balance should consider the winter carbon uptake too.

As we consider the dark treatment, we could point out that the average carbon uptake was more negative where the temperature was higher. *G13* has a three times higher carbon loss than *G6*. Since there is not photosynthesis, we can consider the carbon uptake as an average carbon loss, meaning average respiration. So, this follows the trends that we observed so far and many studies have been shown (Tjoelker *et al*., 1999; Atkin and Tjoelker, 2003; King *et al*., 2006; Kurepin *et al*., 2018).

Species also show a difference in carbon uptake. Contrarily from what we have seen so far, *Picea abies* shows a higher carbon uptake than *Abies alba*. This could be linked to the photosynthetic performances that the plants had during daylight. Nonetheless, in the *dark* treatment *Picea abies* shows a larger carbon loss than *Abies alba*. It means, that according with previous measurements, *Picea abies*, has a larger respiration rate than *Abies alba*. In addition, the difference in carbon uptake shows that *Picea abies* has from 10-50% higher average carbon uptake, it is larger at lower temperatures than higher. However, the large error does not really make these results trustable.

Linear models obtained from these measurements do not evidence any significance effect of temperature in the outdoor experiment. The lines have indeed a very little negative slope. However, *Picea abies* has a higher intercept than *Abies alba*, meaning that it has a slightly higher average carbon uptake. Neither the temperature treatment nor the species are significantly different from each other. On the other hand, there was a negative trend of the lines in the indoor experiment. The latter shows that average carbon uptake decreases exponentially with temperature in both the species. It means that the effect of temperature on carbon uptake is significant. In particular, the effect concerns the temperature treatments at which seedlings were exposed to. The carbon uptake halved with an increase of 8°C. Considering that global warming will cause an increase in average of 4.5°C at northern-latitudes (Hoegh-Guldberg *et al*., 2018), the carbon uptake decrease could be significative.

Species did not show any significant difference between each other. This supports the idea that the variation within the two species that was observed among mean values is too wide to discriminate a significant different behaviour.

In addition, we can point out an increasing variation of responses with an increase of temperature. If we observe the range of values covered by the measurements at different temperature treatments, it is appreciable that the variation in *G13* is the 80% wider than in *G6*. This last point could be very interesting in the climate change scenario. A wider set of responses leads to an increased uncertainty. Meaning that, the projections could be more difficult to be tracked. If the pattern of responses is not clear, we could expect very different behaviours that could obstacle and slow down good management practices. This finding supports the idea that responses to carbon cycle are very uncertain (Settele *et al*., 2014).

Summing up, light treatment influenced carbon uptake, showing larger uptake in ambient light plots. Temperature, instead, had a negative effect on average carbon uptake. None of the treatment caused an average negative carbon uptake in the plots exposed to a certain amount of lights.

Nevertheless, respiration was measured only twice a day, before sunrise and after sunset, this means that the measurements exclude overnight respiration. The latter could lead to a diurnal negative carbon balance. This fact addresses to further measurement to assess a more precise diurnal carbon balance. Finally, plants exposed to darkness follow the expected trend of negative carbon uptake that becomes more negative with warmer temperatures. Species show a small difference from each other with higher carbon uptake and carbon loss in *Picea abies*. We need to point out that average values had a significant standard deviation, meaning that more measurements are needed to clarify what was found.

4.4 Growing season: growth and phenology

4.4.1 Plant growth depends on temperature and light treatments in deciduous trees

Some differences in growth were found in plants subjected to different treatments. In the indoor experiment, where plants had a wider spectrum of temperatures, only *Larix X eurolepis* and *Fagus sylvatica* showed differences of growth related to temperature treatments. However, they have different responses to the treatments. Temperatures in *G13* and *G11* negatively influenced the growth in height of *Larix X eurolepis*. The latter, had a higher growth in the lower temperature treatments, meaning *G9* and *G6*. The highest growth was indeed recorded in *G9* where the plants increased in average more than 150 cm. It has been already reported by Huang *et* al., (2017) that *Larix kaempferi* growth will be negatively influenced by climate warming. In addition, a prolonged exposition to warmer temperatures could cause growth limitation, especially if this is linked with water scarcity (Kharuk *et al*., 2019).

Fagus *sylvatica* appears to take advantage of the temperature instead. The seedlings placed in *G13* and *G11* had a higher growth in height than those placed in the other temperature treatments. In this case, the highest growth was observed in *G11*, with an average of little more than 100 cm, nonetheless, average growth of seedlings placed in *G13* was 100 cm. The other temperature treatments have averages closer to 80 cm. We could distinguish responses above and below 11°C, there is, indeed, a sort of jump between the treatments. Seedlings grew 25% more in treatments above 11°C. There is a connection between the growing season and winter temperatures. They, indeed, seem to delay the end of the growth under milder conditions (Prislan *et al*., 2019). As reported by Signarbieux *et al*., (2017) plants of *Fagus sylvatica* that grew in cooler sites had grown 84% less in volume and 186% higher stem elongation.

Temperature treatments did not have any effect on the growth in height of the other species, and none in growth in diameter. The outdoor experiments results, instead, do not show any effect of temperature treatment in growth of both height and diameter.

Light treatments had an effect only on *Quercus robur*. The latter had different behaviours if the amount of light received during the experimental month is considered. The seedlings show a higher growth in diameter if they were subjected to light instead of total dark treatment, in the indoor experiment. However, they had a lower growth in diameter compared with shaded plants in the outdoor experiment. In the first place, we can deduce that light influenced growth in diameter. Secondly, that there is a different response between indoor and outdoor experiment. *Quercus robur* is not a shade-tolerant species (Kunstler *et al*., 2005), meaning that it should show a lower growth rate in shadow than in light. Full-light exposition should in fact increase the growth rate of *Quercus robur* (Valladares *et al*., 2000). What we observed in our experiment is in contrast with these results.

Shaded plants grew three times more than those exposed to ambient light, in the outdoor experiment, disagreeing what we expected from previous studies. Nevertheless, full-light plants grew 40% more than those subjected to dark treatment, in the indoor experiment, agreeing, this time, with the quoted studies. These contrasting findings can be explained by different responses to the shade level, as already mentioned in Ziegenhagen and Kausch, (1995). Meaning that, *Quercus robbur* could be advantage by a low level of shade and disadvantaged by a high level of it. These findings could limit the northern shifting of *Quercus robur*, meaning that the absence of light could negatively influence the growth of this species. In addition, our study refers to differences in light levels during dormancy and not during growing season, addressing for the necessity of more studies during winter.

All the other plants did not show any effect of light on their growth in both height and diameter. Our results open a new window on the importance to study the effect of light and temperature treatments on growth during dormancy. Evergreen trees did not show any effect on growth.

4.4.2 Phenology

There are interesting findings about the phenological responses of plants after one month of light and temperature treatments. The indoor experiments gave us the chance to study those effects, whereas the outdoor experiment had too much variation within the measurements to perform significant fits of sigmoid curves, even excluding some outliers. So, we will not focus on the discussion of those data in this project.

Data fitted with a sigmoid curve gave homogeneous results for temperature treatments, and different responses for light treatments. All the species subjected to warmer treatments during winter show an advancement of phenology. Specifically, seedlings placed in *G13* had the highest advancement in phenology. Evergreen conifers, namely *Abies alba* and *Picea abies* show major difference between the warmest and the other treatments. Whereas, the difference in treatments is not that evident in *Larix X eurolepis*. Finally, deciduous broad leaves trees, namely *Fagus sylvatica* and *Quercus robur* reveal a lower difference than evergreen conifers among the treatments. However, all the graphs evidence that *G6* sigmoid is the one that is moved the most to the right, meaning that phenological events of those plants result delayed compared to the other greenhouses. The order of other curves is not linear, in some cases plants set in *G9* are shifted more to the left than those set in *G11*.

Our study shows that the temperature treatments at which plants were exposed from January to February had an effect on the timing of phenological events. Especially, plants exposed to warmer treatments result in an advancement of phenology. Meaning that winter temperature influences flushing time (Vitasse *et al*., 2009). These results show the pattern that we expected to see under warmer conditions due to climate change, meaning an advancement of phenological events (Kramer, 1995; Cleland *et al*., 2007; Penuelas *et al*., 2009; Vitasse *et al.*, 2009; Körner and Basler, 2010). Contrarily, more recent studies reveal a different consequence in plant phenology due to warmer winters. Warmer winters seem, instead, to delay phenological events, due to a lack of chilling temperatures required to break endodormancy (Körner and Basler, 2010; Signarbieux *et al*., 2017; Asse *et al*., 2018). However, the degree of advancement and delay is species specific. Beech trees had a higher advancement at cooler conditions. Whether, some species that need lower chilling during winter to stimulate budburst, like *Picea abies*, did not evidence a phenological delay, but rather an advancement at warmer temperatures (Signarbieux et al., 2017; Asse *et al*., 2018).

To better compare the results, it is important to understand the distribution of inflection points of each individual phenological curve. The inflection point corresponds to approximately half of the maximum score, meaning the budburst occurrence. We expected to see the same pattern that was shown by the models that we used to understand the responses of plants divided by treatment. But we found a greater variety among data, so that only *Quercus robur* revealed a significant effect of temperature on the mid flushing day. Therefore, plants exposed to *G13* had the most advance budburst, around the 75th day. Budburst occurred few days later in *G11* and *G9*, whereas it had a delay of 15 days in *G6*, where it happened around the 90th day. So, temperature significantly advanced the budburst in *Quercus robur*.

The other species don't have a significant difference related to temperature, but some patterns can be seen. Differences between the warmest and the coldest greenhouses, namely *G13* and *G6*, are ≈+4 days for *Abies alba*, ≈+ 14 days for *Picea abies*, 0 days *Larix X eurolepis* and ≈+7 days for *Fagus sylvatica*. We can deduce that the most affected plants by the temperature treatments, in terms of phenology advancement, are *Quercus robur* and *Picea abies*, although the responses have more variability in the latter. Our results agree with the fact that the first species is very sensible to temperature effect (Vitasse *et al*., 2009) and that the second show a phenological advancement during warmer winters (Asse *et al*., 2018). Since responses have similar trends but very different range between days of advancement and delay, it is important to study species-specific response to temperatures (Vitasse *et al*., 2009).

Indoor plants revealed different responses to light treatment in some species. The sigmoid curves of *Picea abies* and *Quercus robur* evidenced discrepancies in day number for certain phenological events. Whereas, the other species did not show any evidence of light treatment in the shape and position of the curves. Seedlings of the evergreen conifer exposed to ambient light treatment flushed earlier than those exposed to full darkness. The same behaviour was observed in *Quercus robur*, with a slightly lower difference than *Picea abies*. These evidences were also supported by the results found in the comparing of mid flushing day, where plants subjected to total darkness treatment showed budburst delayed of ≈+ 10 days for *Picea abies* and ≈+ 9 days for *Quercus robur*. These findings are interesting in the context of north pole ward moving of species due to climate change (Settele *et al*., 2014). If in the future these species will be able to survive to polar conditions, there could be mismatches in the phenology of some of them, like *Picea abies* and *Quercus robur* in terms of delay in the budburst, due to the absence of light during winter. On the other hand, other species could not be affected by darkness, meaning that they could adapt better at dark and warm conditions (Royer *et al*., 2005).

Phenology is influenced by winter temperature and winter light exposure. The beginning of growing season is leaded by daylength, degree of winter chilling and temperature in the days before the flushing (Vitasse *et al*., 2009; Körner and Basler, 2010, Visser and Gienapp, 2019). As we have seen, shift in the phenology is species-specific (Visser and Gienapp, 2019). In addition, it influences the length of growing season (Penuelas *et al*., 2010). Warmer winters seem to have a major effect on autumn phenology than early spring (Suonan *et al*., 2017), with a consistent legacy between spring and autumn events, especially at cooler conditions (Signarbieux *et al*., 2017). Therefore, an earlier spring can be followed by an earlier autumn senescence, meaning that future models should include spring phenology events to forecast autumn phenology events (Keenan and Richardson, 2015). Considering that the advancement of phenological events will be greater at northern latitudes (Liu *et al*., 2019), further studies are needed to better model phenological response of species in climate change scenario.

5. Conclusion

Temperature and light treatments had an effect on plants performances, growth and phenology. Major effects were seen in the indoor experiment, where plants could experience a much higher difference of temperature than in the outdoor one.

Warm winter temperatures negatively affect photosynthesis and respiration. Conifers were not improving their performances under milder conditions. Net photosynthesis was lower for plants grown at warmer temperatures, and the temperature optimum was not matching the growth temperature. It revealed a scarce acclimation of conifers to mild winter temperature. In addition, very low light level limited photosynthesis. Respiration in the dark showed a correlation with temperature treatment.

The models evidenced a exponential increasing of respiration in the dark due to growth temperature and temperature in the moment of measurement. So, we could say that it is important to consider both the average growth temperature and the daily temperature to understand properly the trend of respiration in future climate change scenarios. *Picea abies* and *Abies alba* had different level of respiration, a markedly higher respiration was recorded in the first one.

A very small difference between the two species was also seen in the carbon uptake levels in different treatments. *Picea abies* enhanced the respiration rate in the dark faster, and it showed a slightly higher carbon uptake than *Abies alba*. These differences were flattened in the carbon uptake model, were no difference between the two species was seen as response of growth temperature.

Carbon uptake was found to decrease exponentially with growth temperature of seedlings, addressing more studies to understand if respiration is the only cause of this decreasing or if there are other reasons. In addition, the variation among measurements was also wider with warmer temperature treatment. Meaning we expect to see a widespread set of responses, making more difficult future projections under global warming.

Growth in height was influenced by temperature in different ways, namely *Larix X eurolepis* had negative effect of high temperatures in winter and *Fagus sylvatica* was instead advantaged by warmer winters, both of them showed an effect for the growth in height but not in diameter. On the other hand, *Quercus robur* exposed to dark treatment had a lower growth in diameter than ambient light, despite, it showed an enhanced growth in diameter in the shaded treatment than ambient light. This species could have very different responses (Morin *et al*., 2010). Evergreen conifers didn't show any significant difference in the growth if subjected to low o light or darkness, or with warmer or cooler temperatures.

Phenology was contrarily high influenced by winter temperature. All the plants that grew at warmer temperatures had an advancement in phenology comparing with cooler temperatures. The number of days was strictly related to the species. However, *Quercus robur* and *Picea abies* had the higher number of days of advancement. In addition, winter darkness seemed to drive certain species through a delay in spring phenology events, in particular *Picea abies* and *Quercus robur*. Phenology mismatches due to warming winters are very likely to happen. As we have seen not all the species are affected in the same way by physical conditions (Visser and Gienapp, 2019). Further studies are needed to understand populations dynamics in forest ecosystem.

This study shows that warmer winters have significant effect on winter performances in terms of carbon uptake and respiration, addressing to more studies needed to calculate carbon balance during winter months and to assess global scale carbon trend under climate warming. In addition, we have seen an effect of warmer temperatures on both growth and phenology, showing that it is important to include winter climate in future modelling to better understand the effect of climate change. Nevertheless, light had interesting effects on growth and phenology, meaning that it is important to comprehend darkness tolerance for species that will move pole ward under high latitude warming, especially for long term forest management and planning.

Not all the species react in the same way to global warming. Those that are weaker and less resilient need lot of attention under this dramatic scenario. Biodiversity should be preserved and in order to do that we need to take into account all the multifaced and complex aspects of ecosystems. Therefore, many efforts are needed to better comprehend this complicated issue that nature has been facing. All the responses are incredibly important to lead the best decisions for living organisms and ecosystem survival, and most important, functioning.

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Annex I – Temperature curves

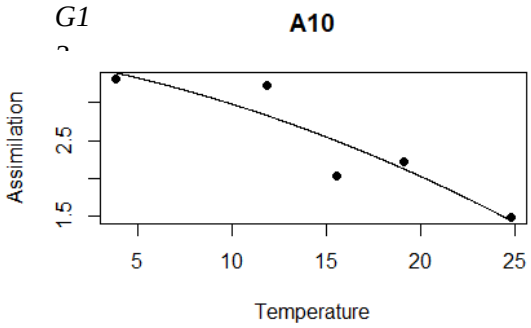
First letter stands for species, 'A' for *Abies alba* and 'N' for *Picea abies*, and the number is an identification of the individual. *G13* and *G6* indicate the greenhouse.

Table I – Temperature optimums (T_{opt}) calculated for each individual under different light levels.

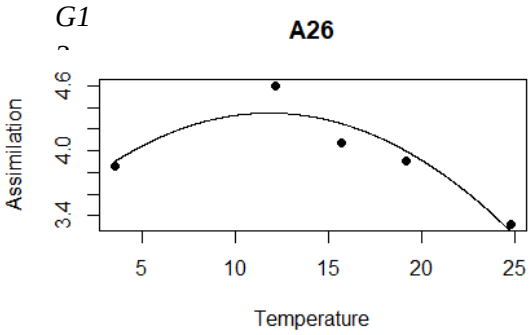
Plot	Individual	Temperature Treatment	T_{opt} (°C)	PAR (µmol m^{-2} s^{-1})
G13	A10	*G13*	3.90	100
G13	A68	*G13*	3.90	100
G13	N123	*G13*	4.10	100
G13	N139	*G13*	5.02	100
G13	N134	*G13*	6.14	100
G13	N140	*G13*	6.94	100
G13	A26	*G13*	11.84	100
G6	N119	*G6*	4.95	100
G6	N127	*G6*	10.02	100
G6	A39	*G6*	10.95	100
G6	A30	*G6*	14.39	100
G13	A10	*G13*	4.00	200
G13	N134	*G13*	8.77	200
G13	N139	*G13*	9.63	200
G13	N140	*G13*	11.72	200
G13	A68	*G13*	11.82	200
G13	A26	*G13*	12.62	200
G13	N123	*G13*	12.87	200
G6	A39	*G6*	12.37	200
G6	N119	*G6*	15.92	200
G6	N127	*G6*	17.32	200
G6	A30	*G6*	22.41	200

Temperature curves performed at 100 µmol m^{-2} s^{-1}

G1



G1



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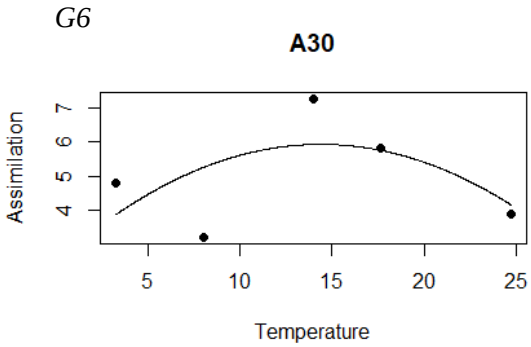

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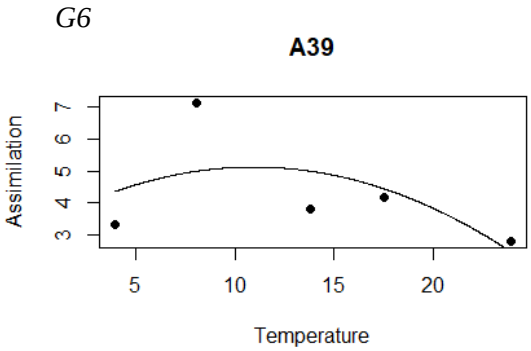

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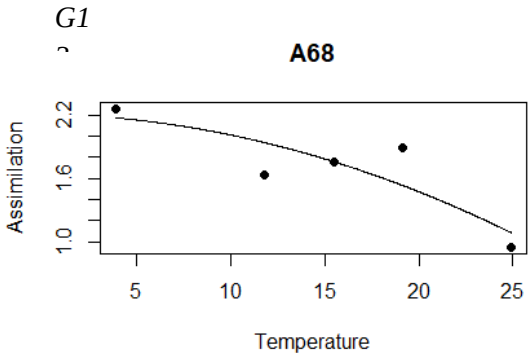

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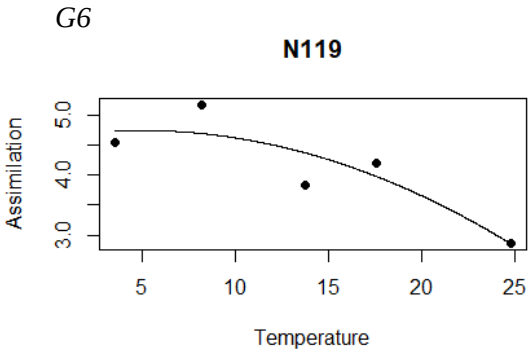

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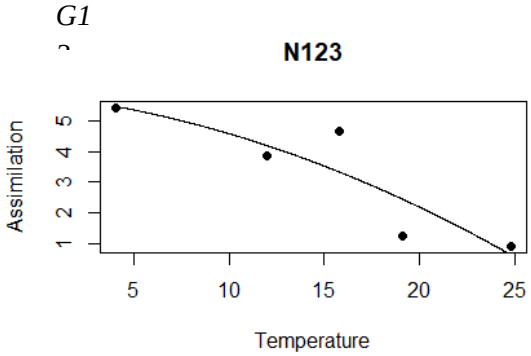

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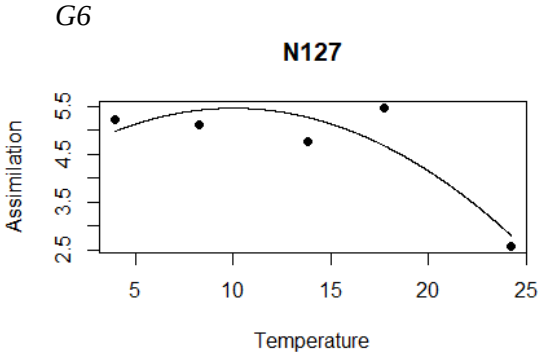
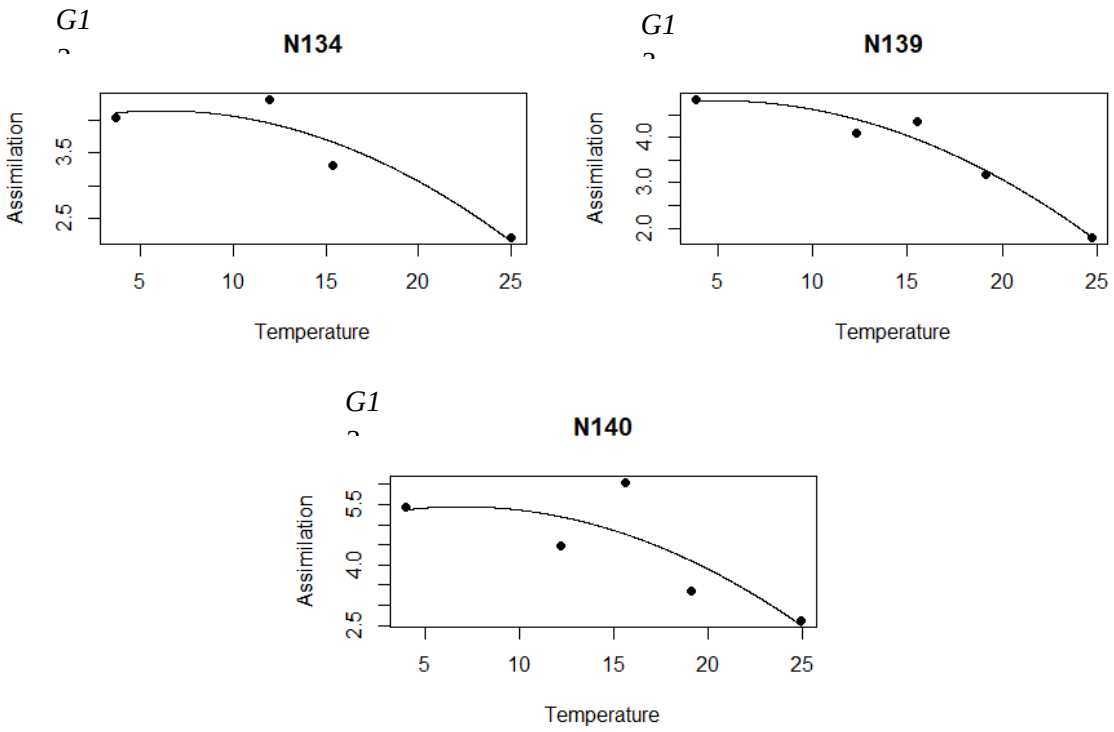
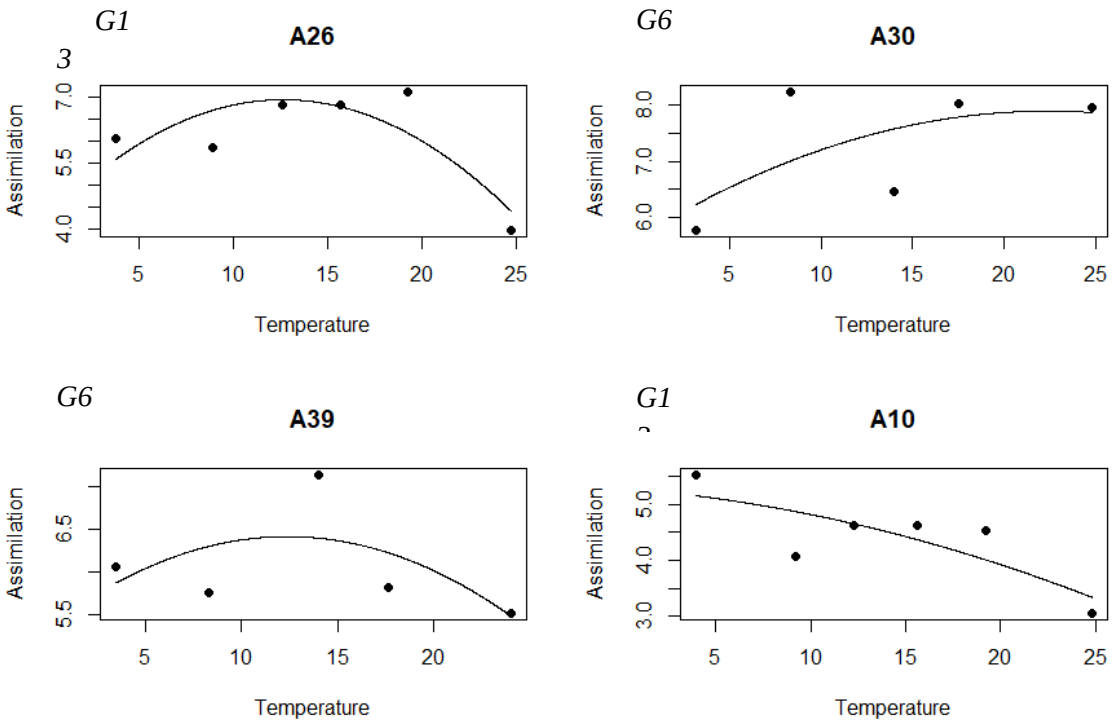
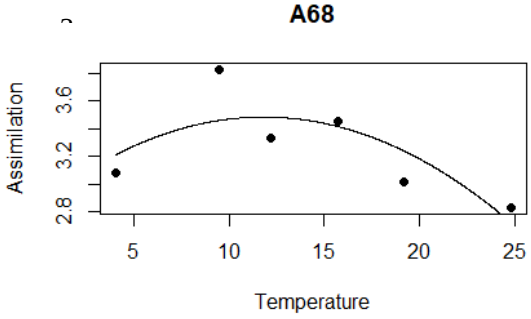




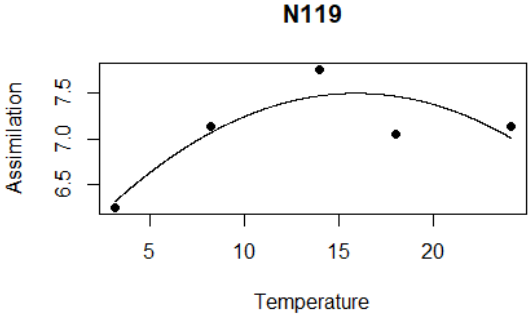
Temperature curves at 200 µmol m^{-2} s^{-1}



G1



G6



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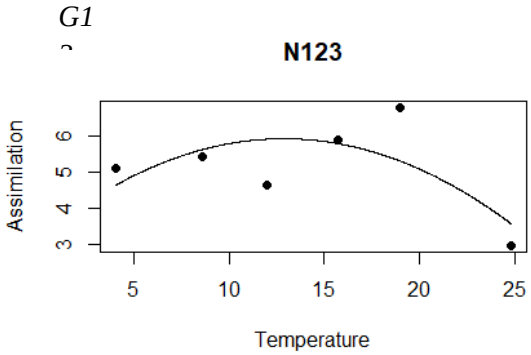
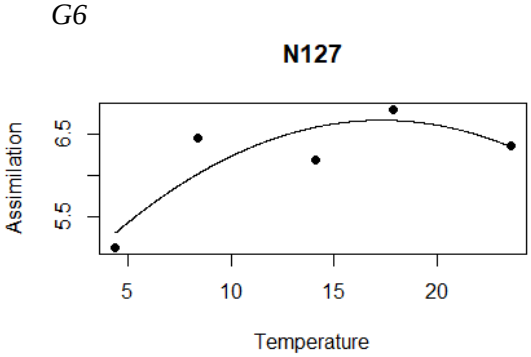
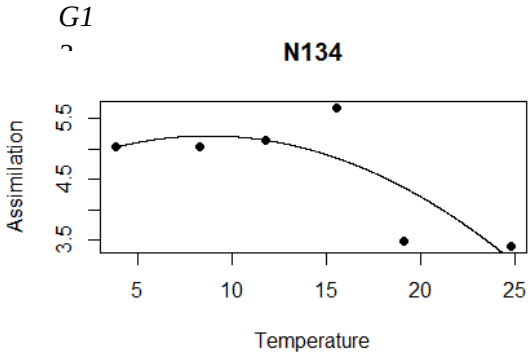


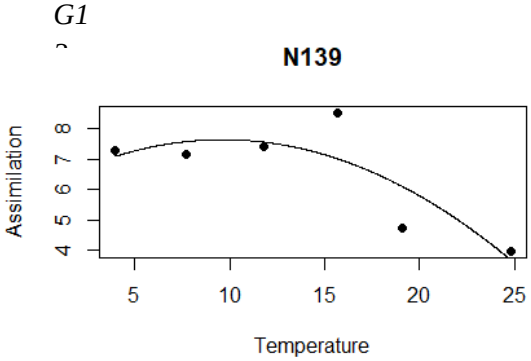
G6



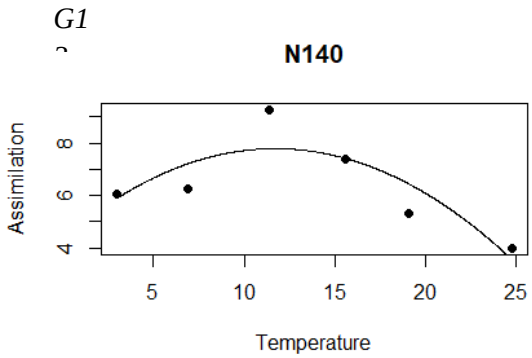
G1



G1

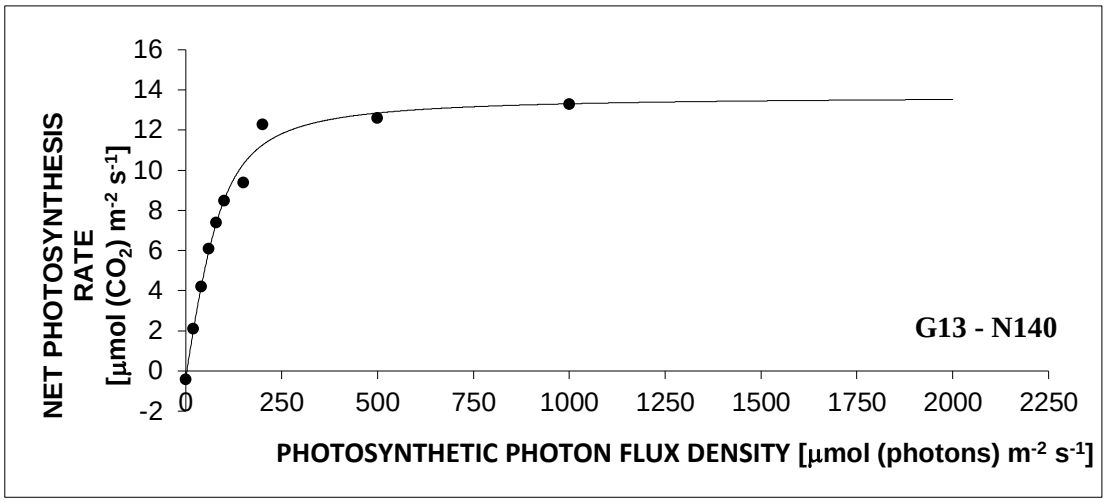


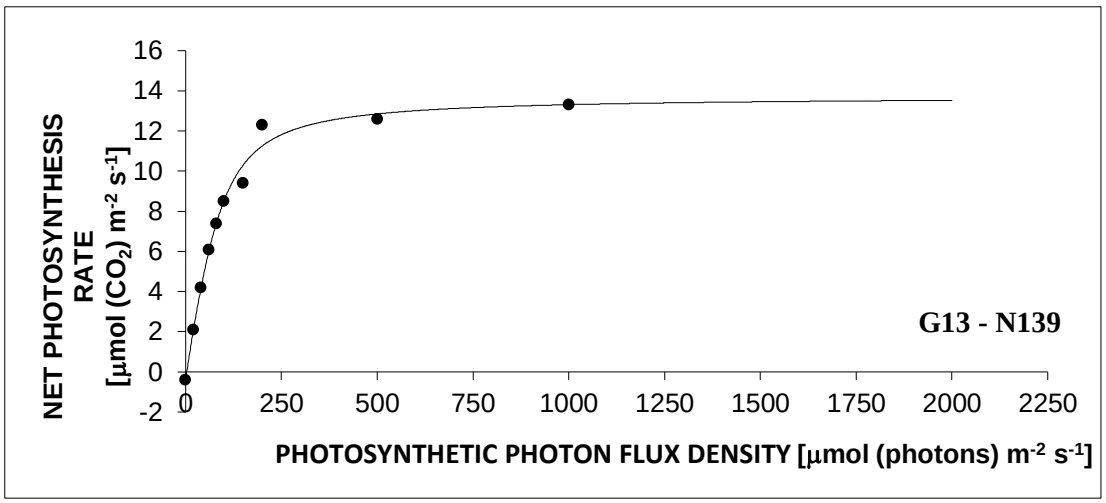
G1

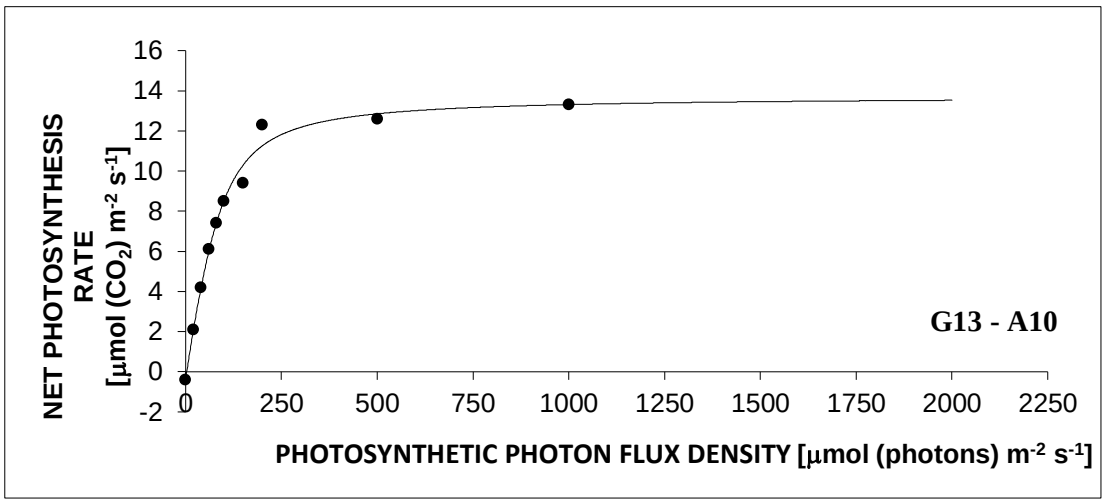


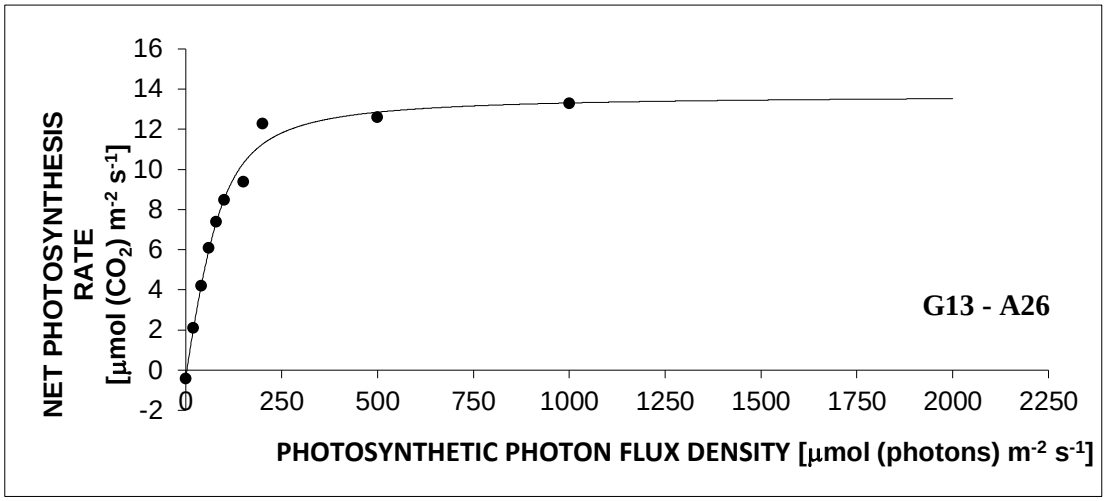
Annex II – Light curves

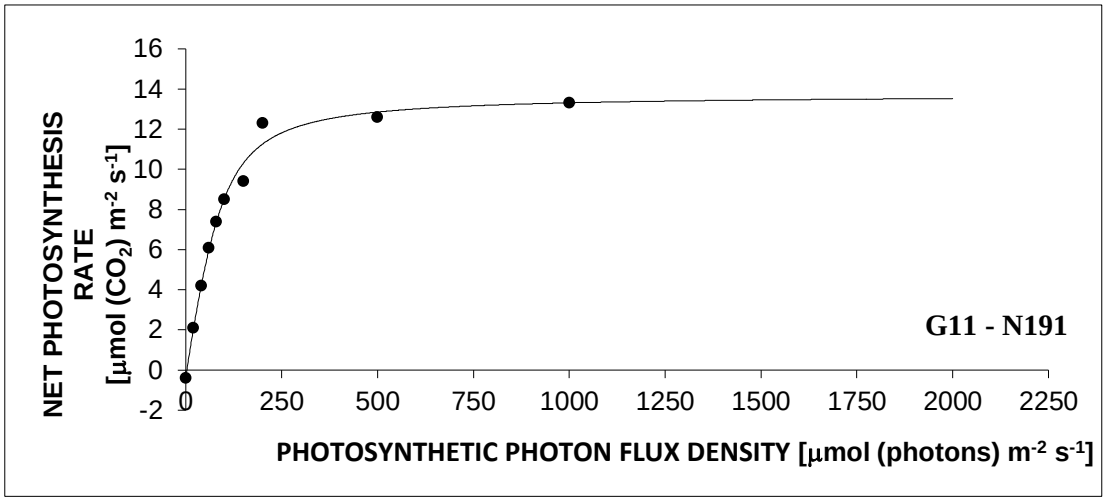
Light curves fitted with Lobo *et al*., 2013.

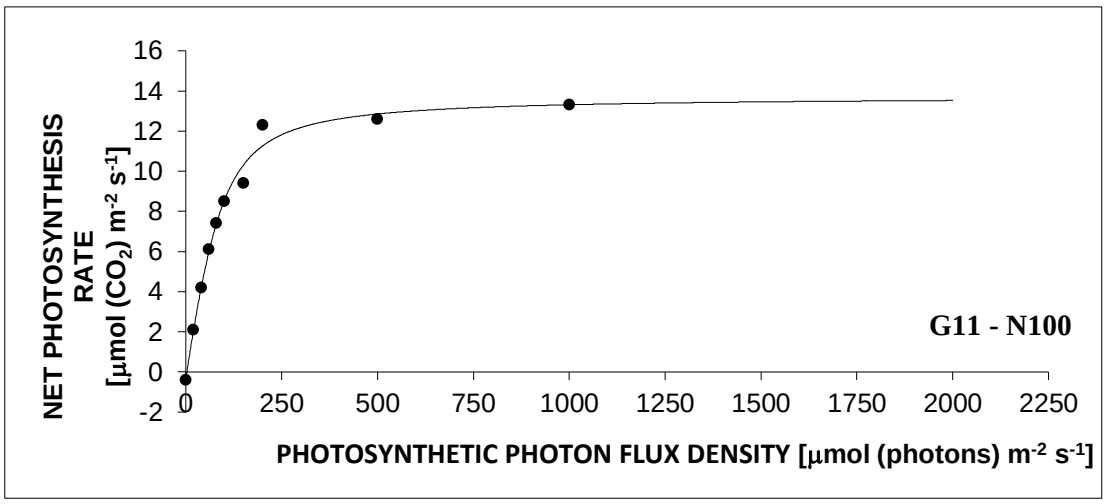


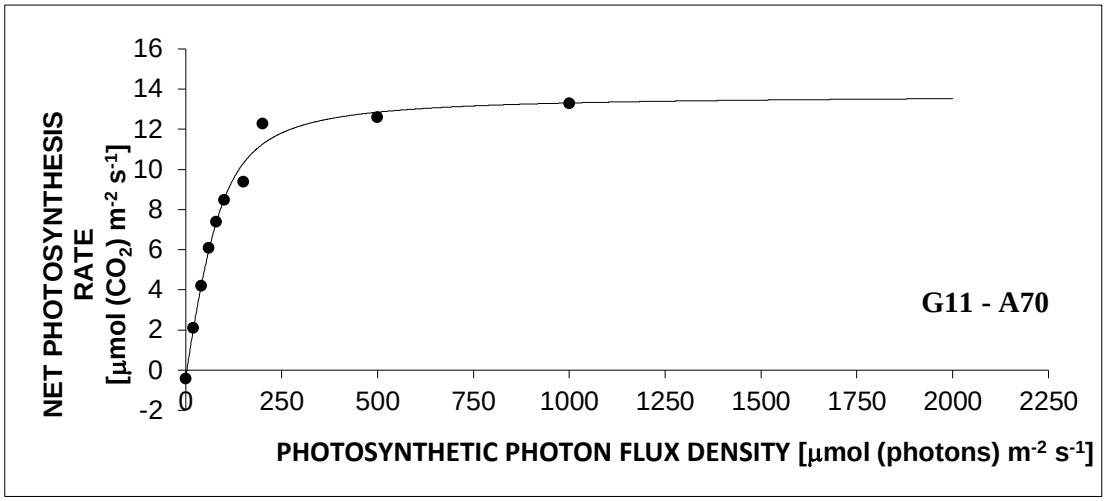


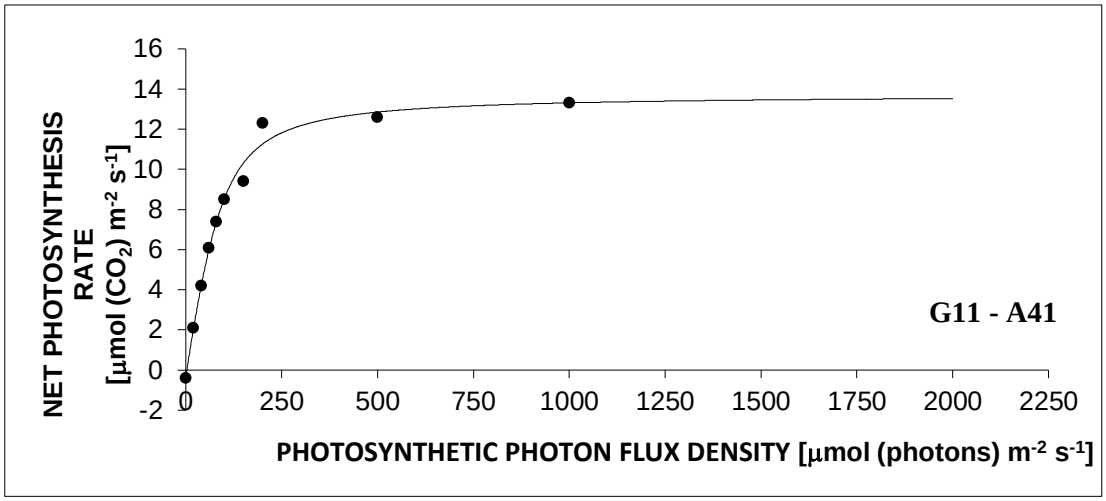


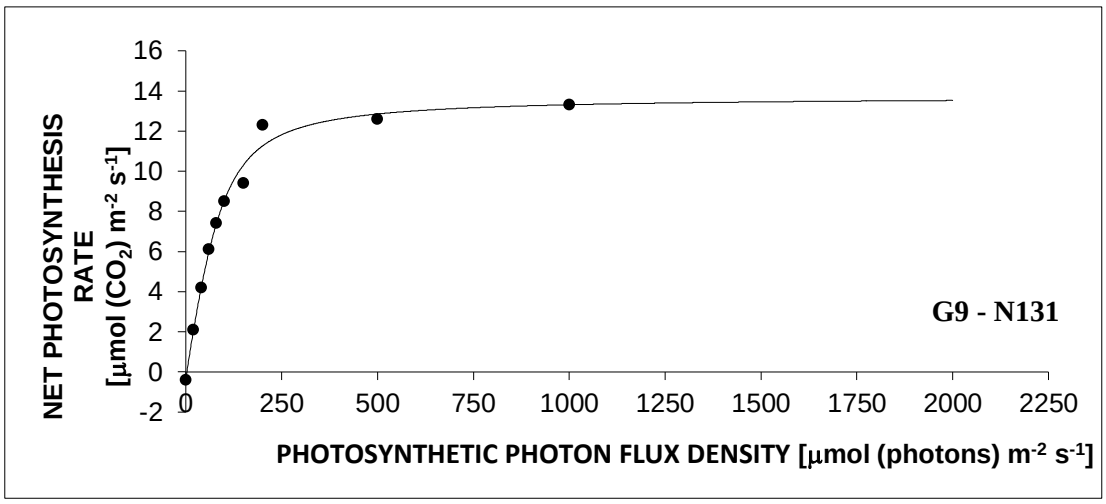

NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G13 - A26
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]

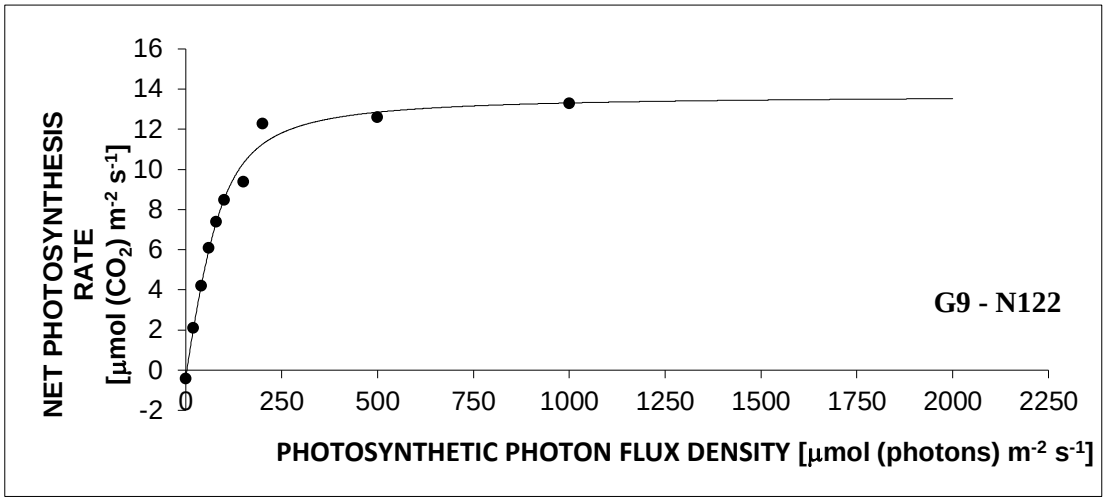

NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G11 - N191
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]

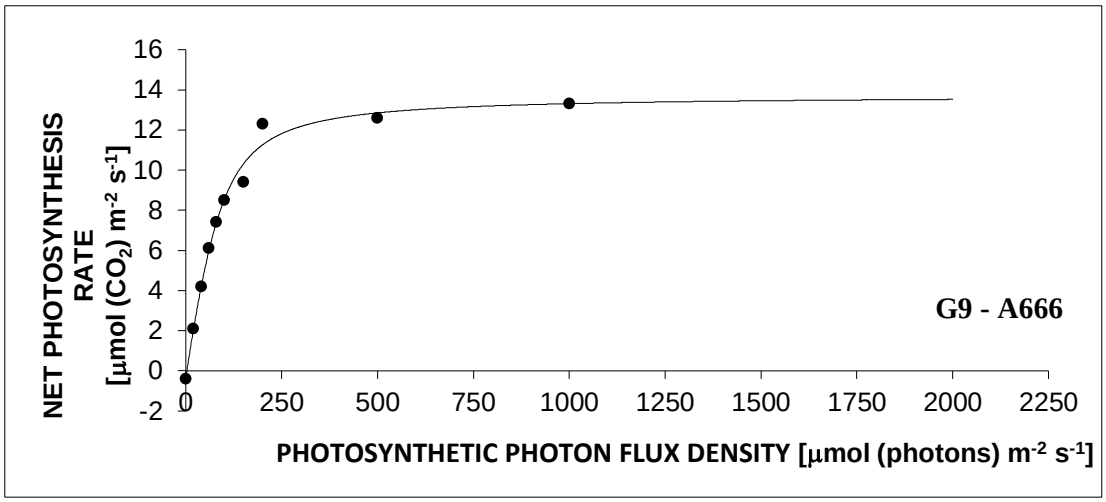

NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G11 - N100
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]

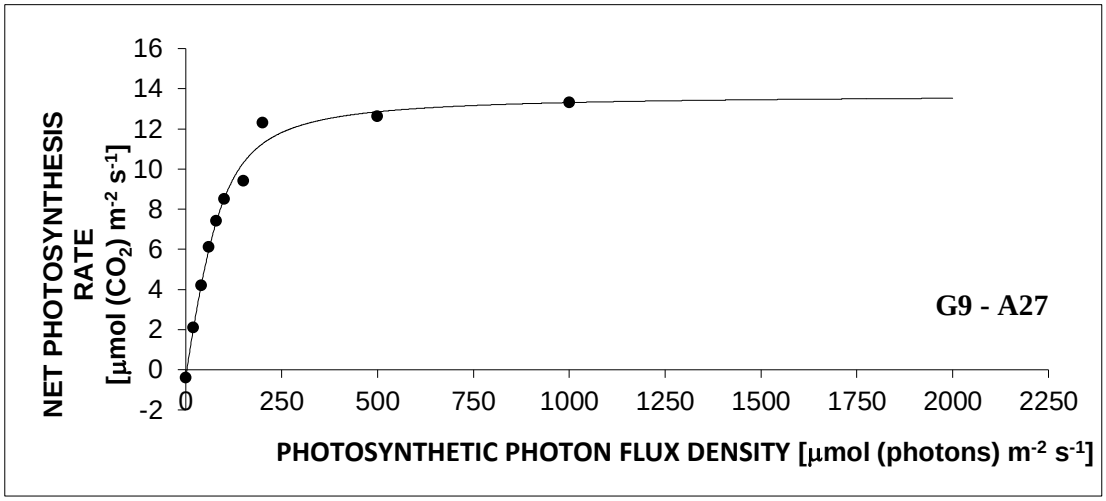

NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G11 - A70
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]

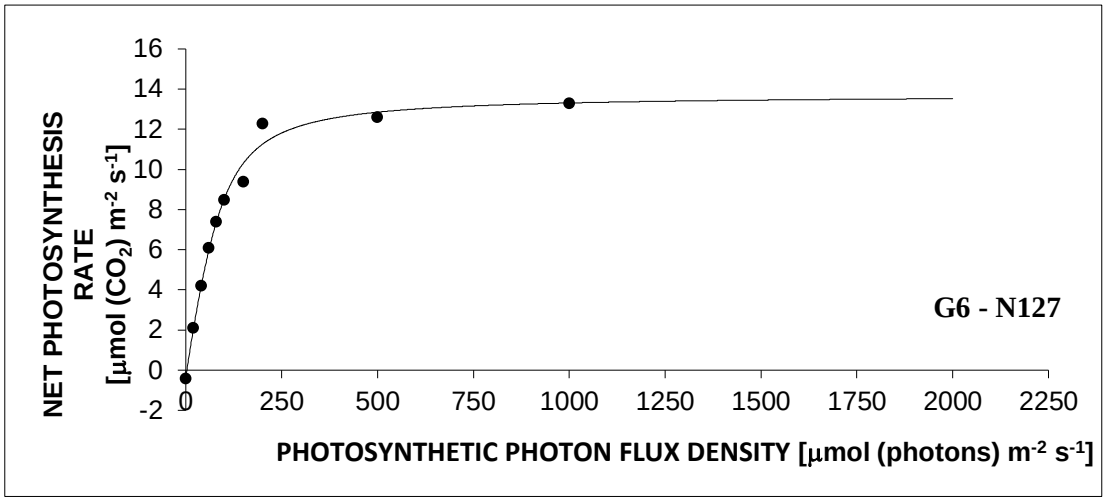

NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G11 - A41
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]

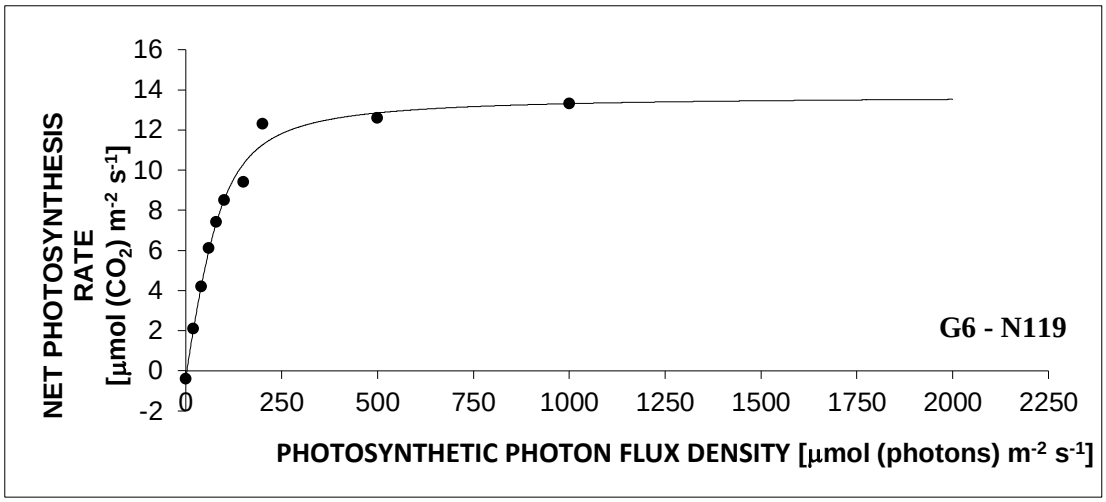

NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G9 - N131
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]

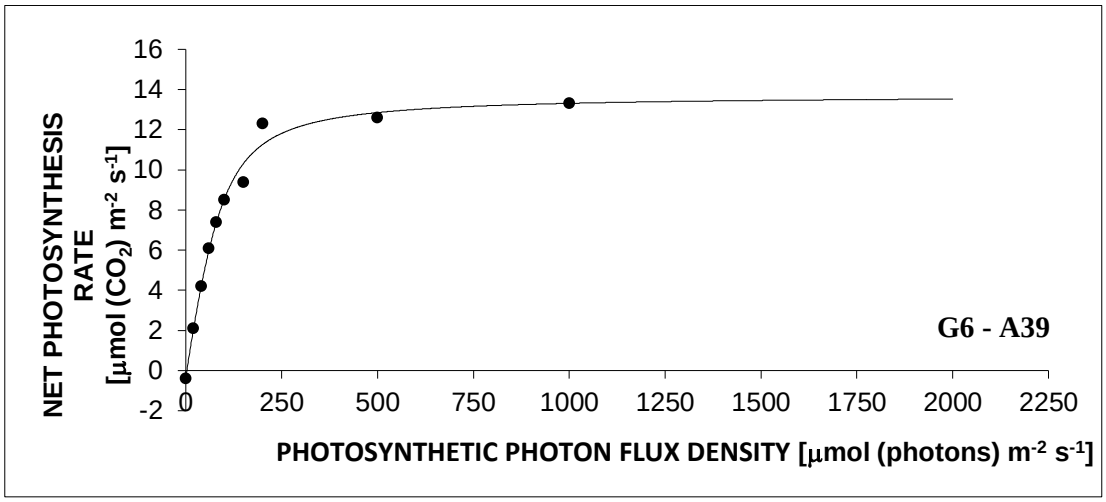

NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]
G9 - N122


NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]
G9 - A666


NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]
G9 - A27


NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G6 - N127
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]


NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G6 - N119
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]


NET PHOTOSYNTHESIS RATE [μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G6 - A39
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]

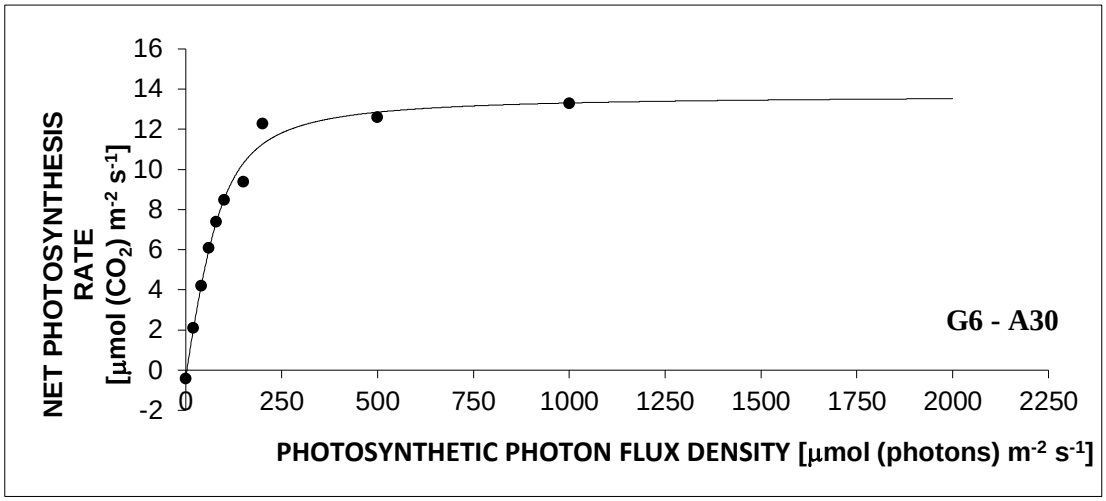
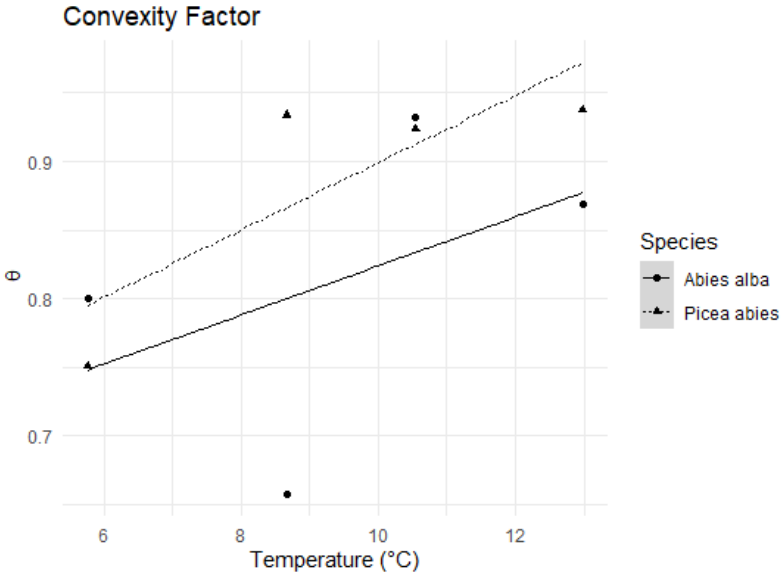
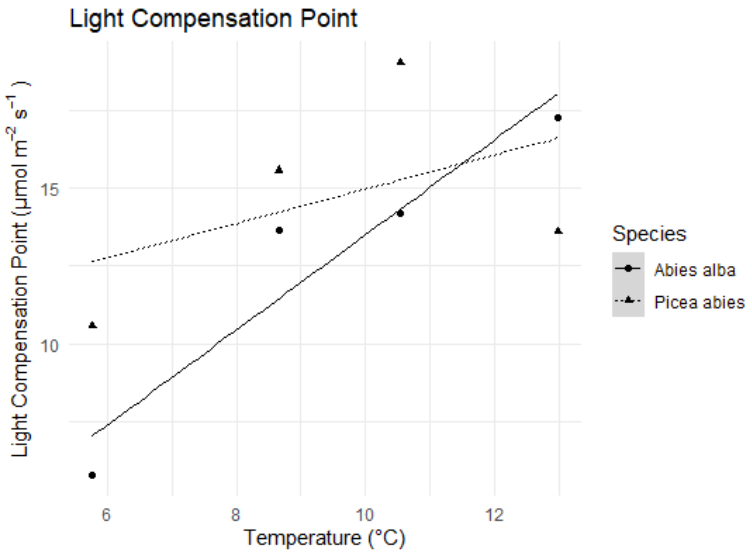

NET PHOTOSYNTHESIS RATE
[μmol (CO2) m-2 s-1]
16
14
12
10
8
6
4
2
0
-2
0
250
500
750
1000
1250
1500
1750
2000
2250
G6 - A30
PHOTOSYNTHETIC PHOTON FLUX DENSITY [μmol (photons) m-2 s-1]

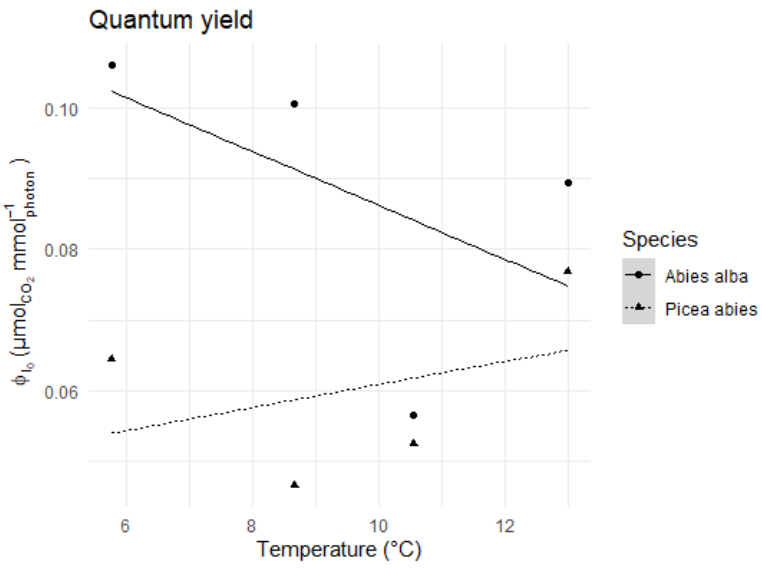
Table II – Calculated variables divided per individual and plot. First letter of the individual name stands for the species ('N' refers to *Picea abies* and 'A' refers to *Abies alba*).

Plot	Individual	$\varphi_{(Io)}$ µmol (CO2) $mmol^{-1}$ (photons)	P_{gmax} µmol (CO2) m^{-2} s^{-1}	θ	R_D µmol (CO2) m^{-2} s^{-1}	LCP µmol (photons) m^{-2} s^{-1}
G13	**N140**	0.09	10.25	0.91	1.16	13.16
G13	**N139**	0.06	8.59	0.97	0.89	14.00
G13	**A10**	0.06	9.46	0.78	1.27	22.65
G13	**A26**	0.12	14.88	0.95	1.43	11.88
G11	**N191**	0.04	5.57	0.94	0.98	26.23
G11	**N100**	0.07	10.54	0.91	0.79	11.85
G11	**A70**	0.07	14.11	0.98	0.87	11.73
G11	**A41**	0.04	8.85	0.89	0.65	16.62
G9	**N131**	0.05	9.16	0.94	0.60	13.17
G9	**N122**	0.05	8.91	0.93	0.84	17.97
G9	**A666**	0.16	17.42	0.79	1.29	8.35
G9	**A27**	0.04	9.95	0.53	0.80	18.95
G6	**N127**	0.07	7.44	0.75	0.68	9.99
G6	**N119**	0.06	8.71	0.75	0.65	11.13
G6	**A39**	0.09	10.33	0.85	0.74	8.79
G6	**A30**	0.13	14.07	0.75	0.34	2.73

Parameters calculated with the light curves and plotted as a function of temperature treatment. No significant effect of temperature was found in any of them.

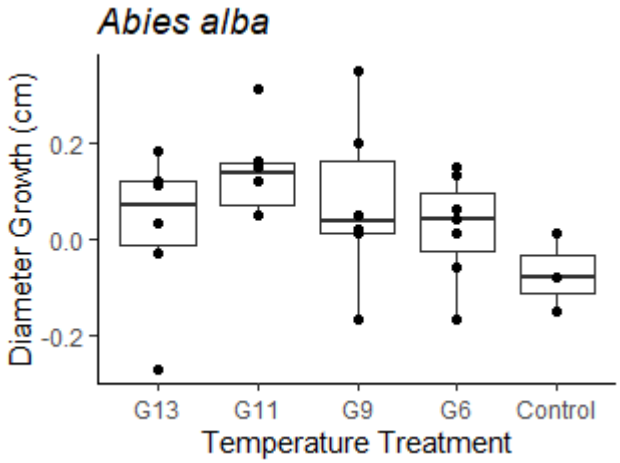


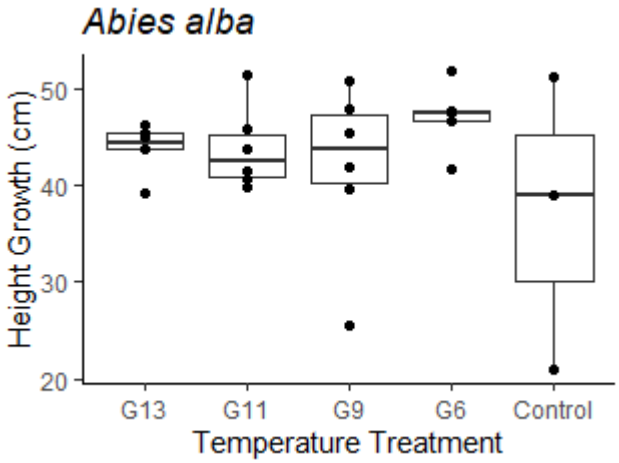


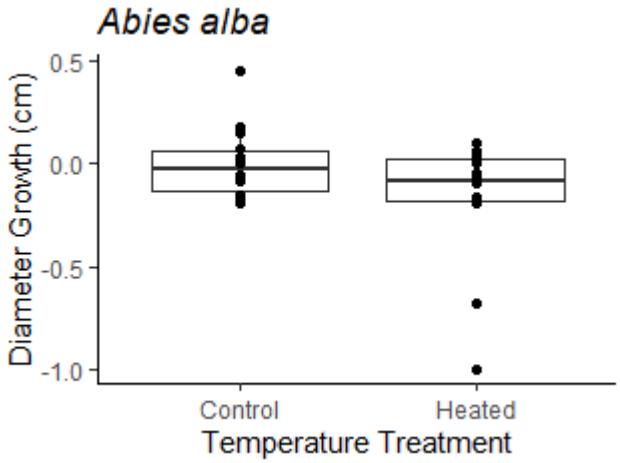


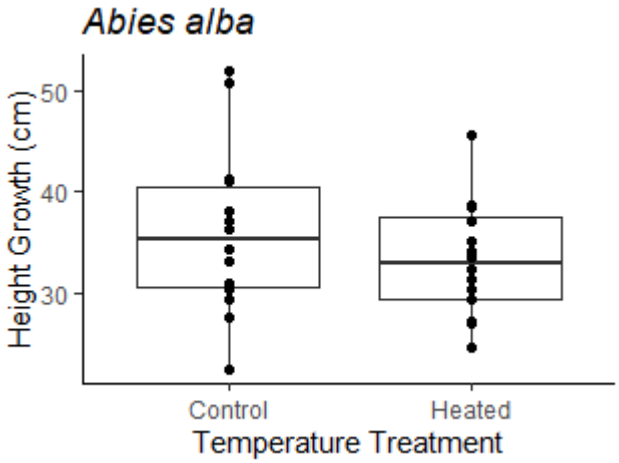
Annex III – Growth

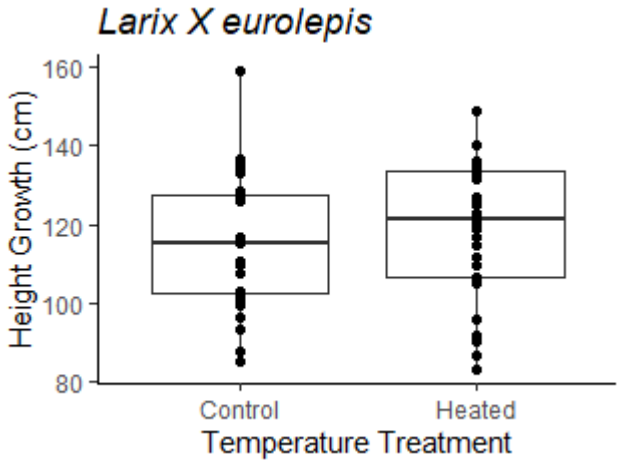
Divided by temperature treatment.

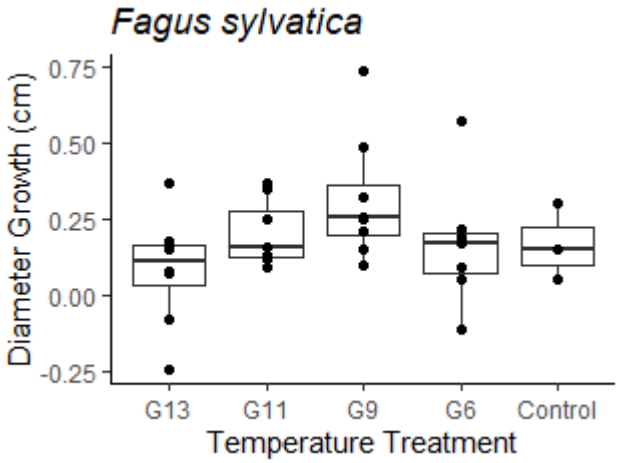


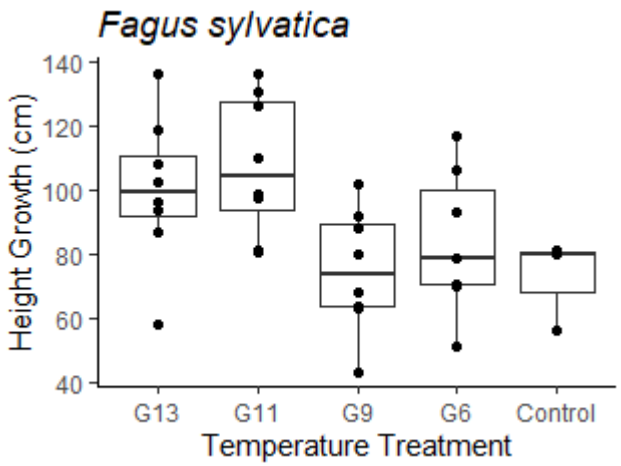


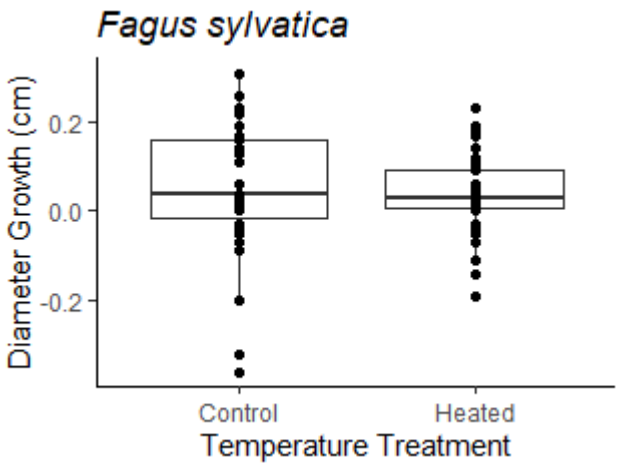


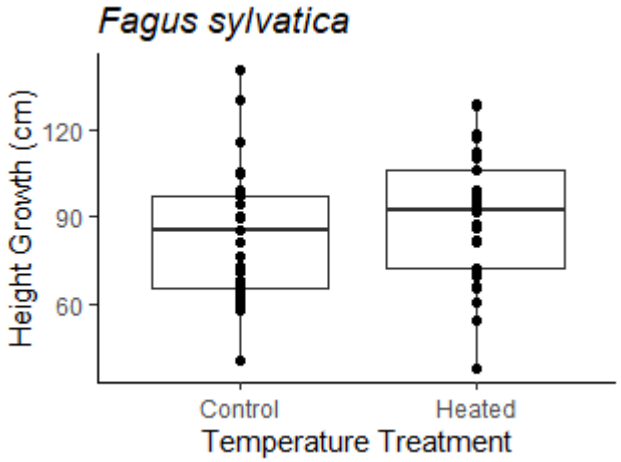


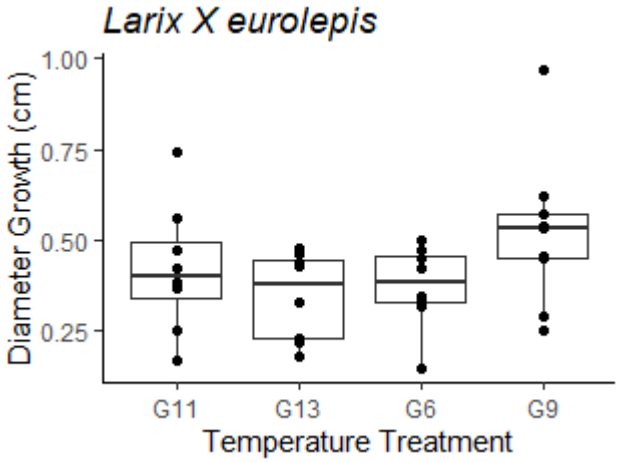


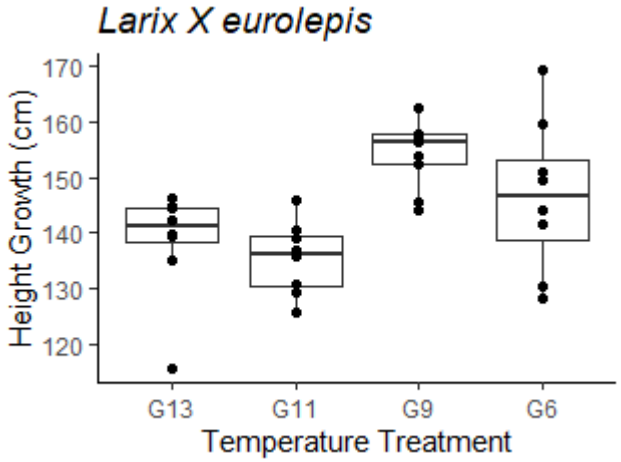


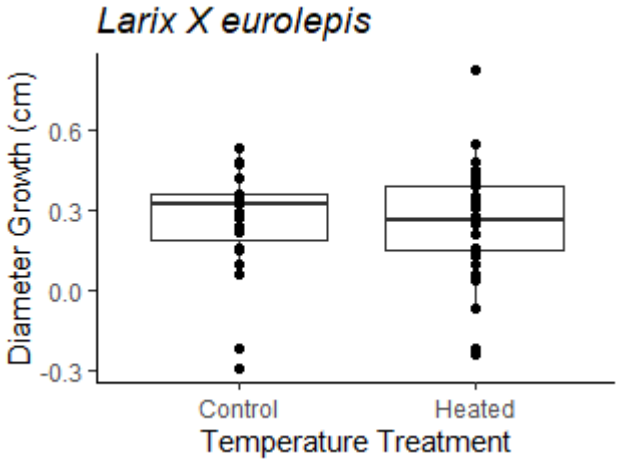

Fagus sylvatica
Height Growth (cm)
140
120
100
80
60
40
G13
G11
G9
G6
Control
Temperature Treatment

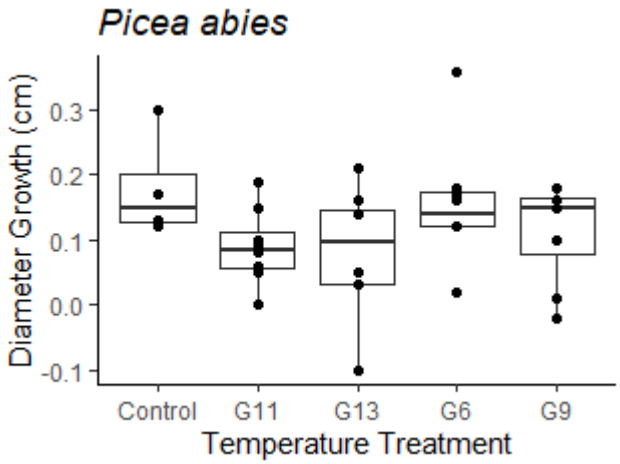

Fagus sylvatica
Diameter Growth (cm)
0.2
0.0
-0.2
Control
Heated
Temperature Treatment

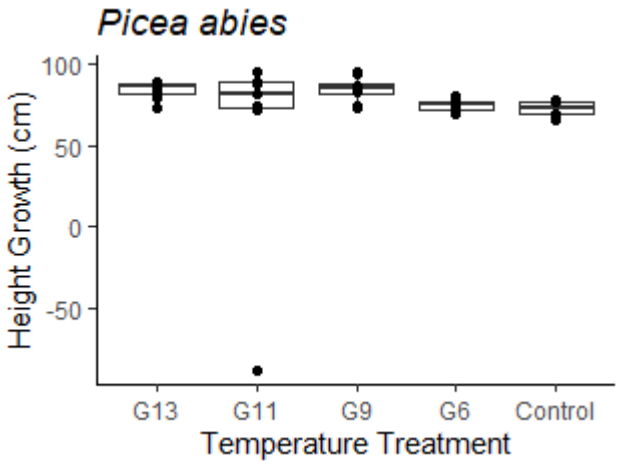

Fagus sylvatica
Height Growth (cm)
120
90
60
Control
Heated
Temperature Treatment

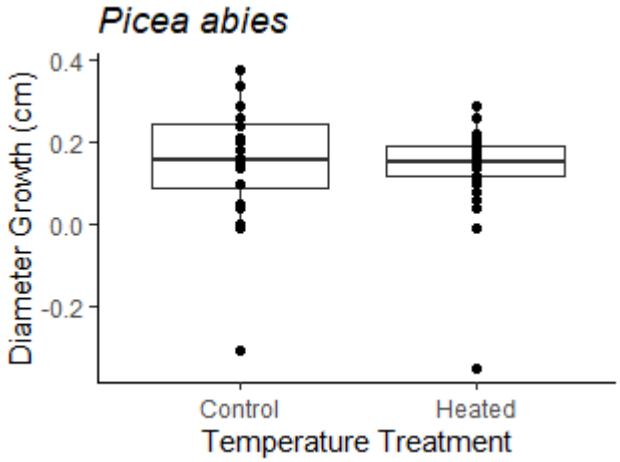

Larix X eurolepis
Diameter Growth (cm)
1.00
0.75
0.50
0.25
G11
G13
G6
G9
Temperature Treatment

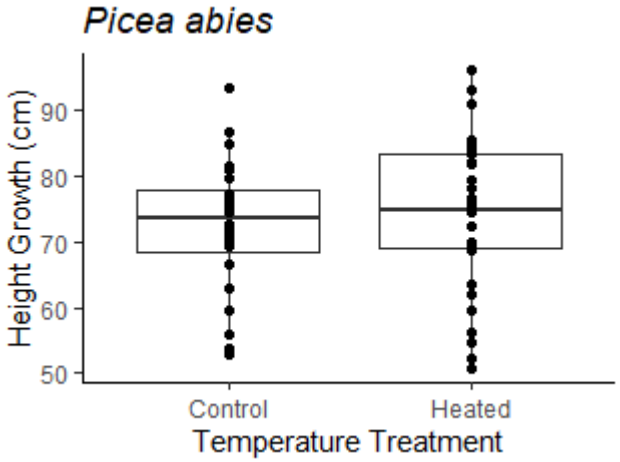

Larix X eurolepis
Height Growth (cm)
170
160
150
140
130
120
G13
G11
G9
G6
Temperature Treatment

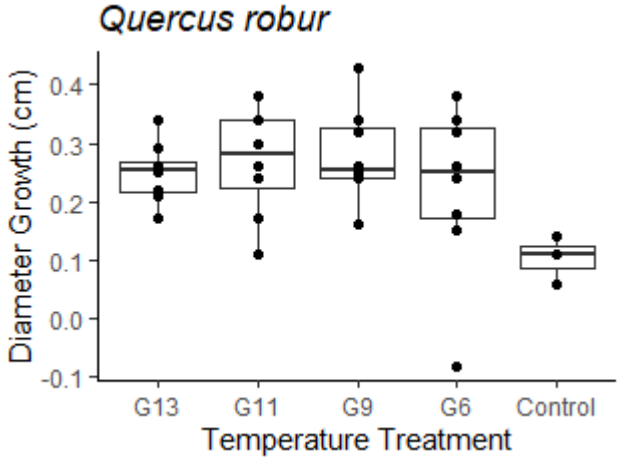

Larix X eurolepis
Diameter Growth (cm)
0.6
0.3
0.0
-0.3
Control
Heated
Temperature Treatment

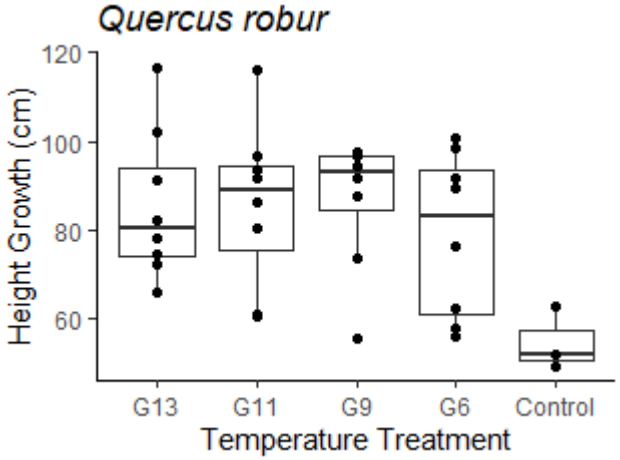

Picea abies
Diameter Growth (cm)
0.3
0.2
0.1
0.0
-0.1
Control
G11
G13
G6
G9
Temperature Treatment

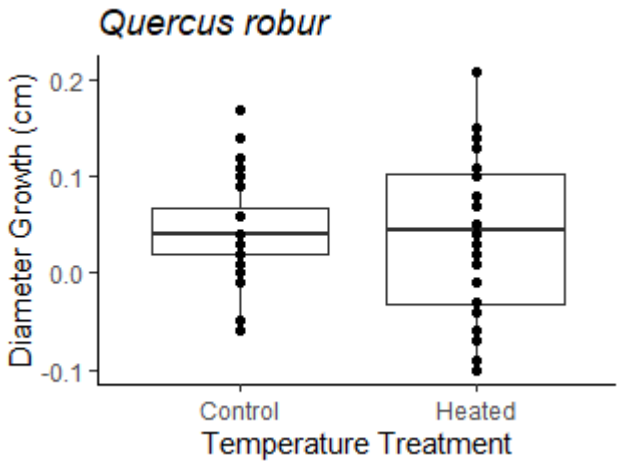

Picea abies
Height Growth (cm)
100
50
0
-50
G13
G11
G9
G6
Control
Temperature Treatment

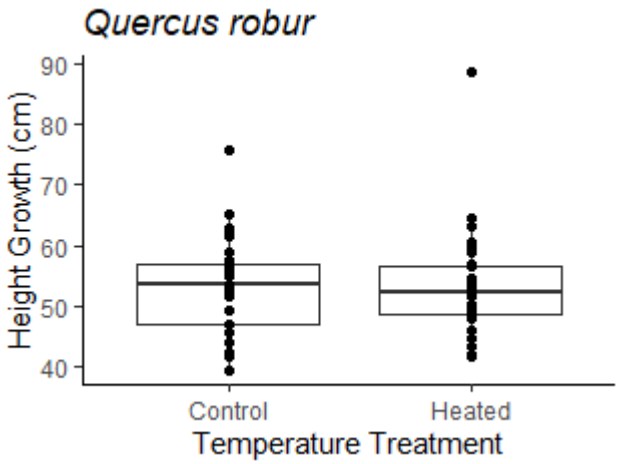

Picea abies
Diameter Growth (cm)
0.4
0.2
0.0
-0.2
Control
Heated
Temperature Treatment


Picea abies
Height Growth (cm)
90
80
70
60
50
Control
Heated
Temperature Treatment

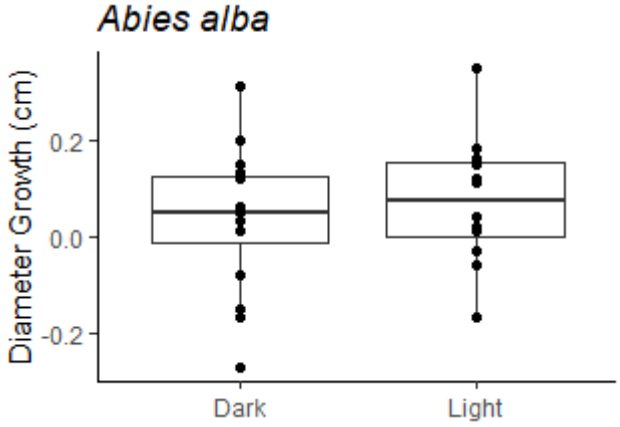

Quercus robur
Diameter Growth (cm)
0.4
0.3
0.2
0.1
0.0
-0.1
G13
G11
G9
G6
Control
Temperature Treatment

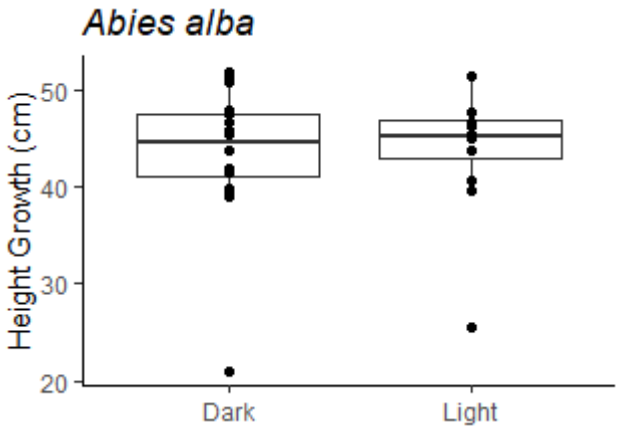

Quercus robur
Height Growth (cm)
120
100
80
60
G13
G11
G9
G6
Control
Temperature Treatment

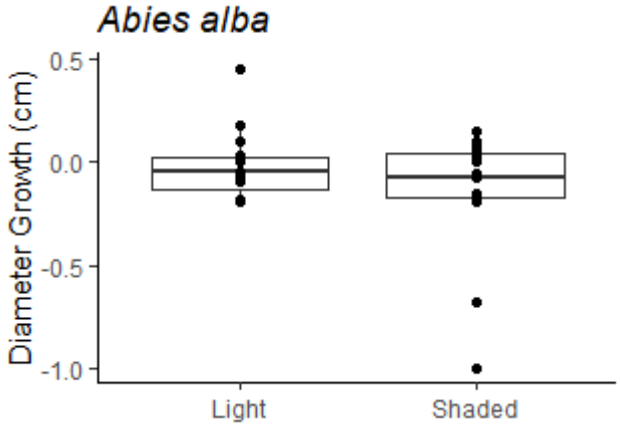

Quercus robur
Diameter Growth (cm)
0.2
0.1
0.0
-0.1
Control
Heated
Temperature Treatment

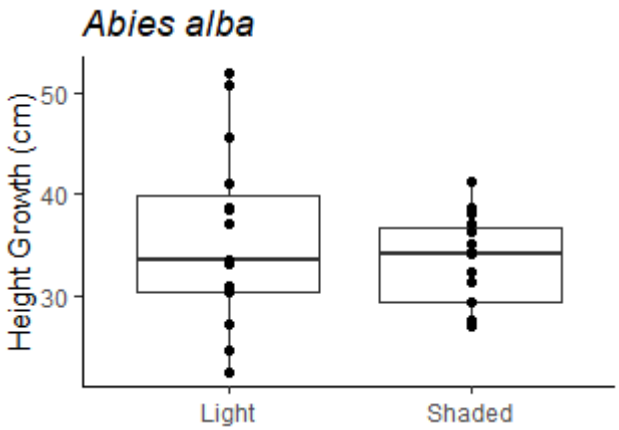

Quercus robur
Height Growth (cm)
90
80
70
60
50
40
Control
Heated
Temperature Treatment

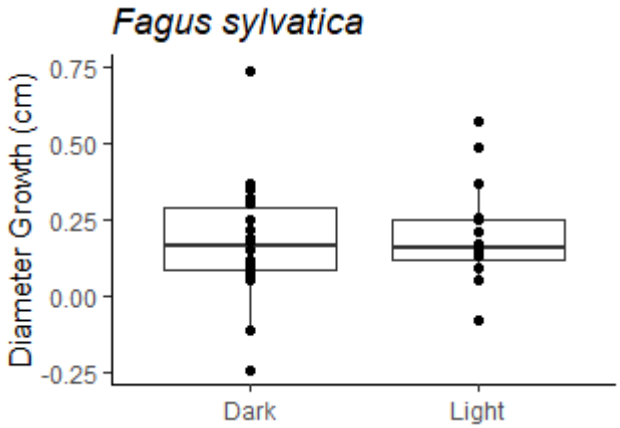
Growth analysis divided by light treatment.

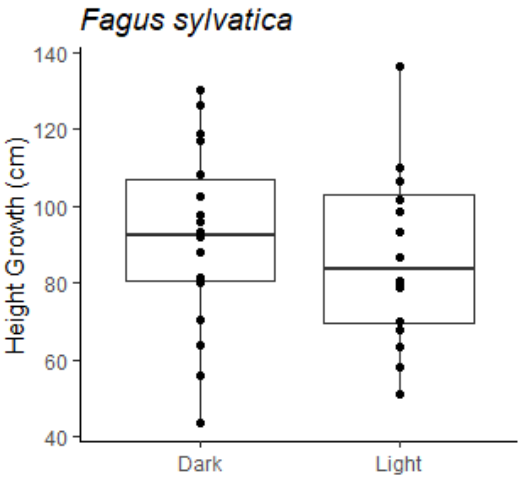


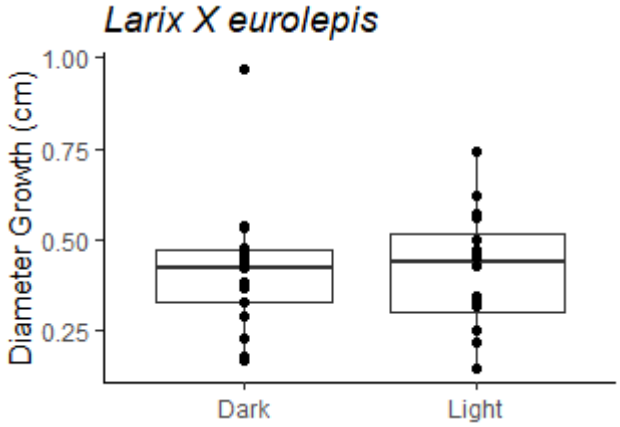


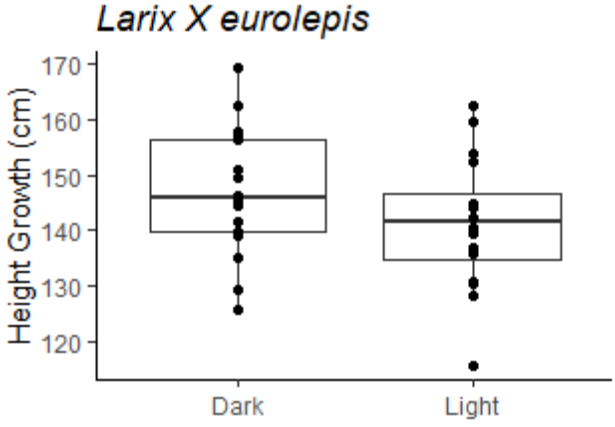


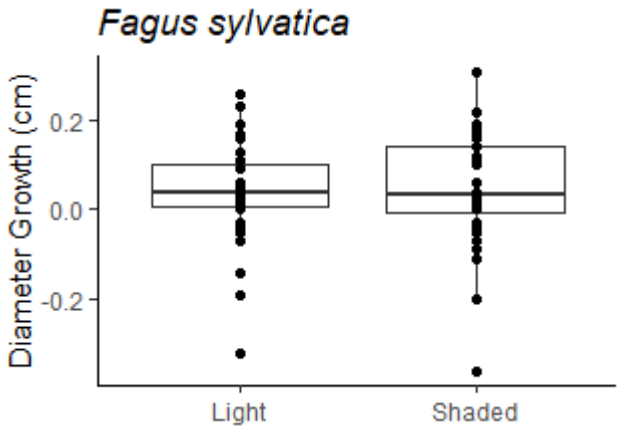


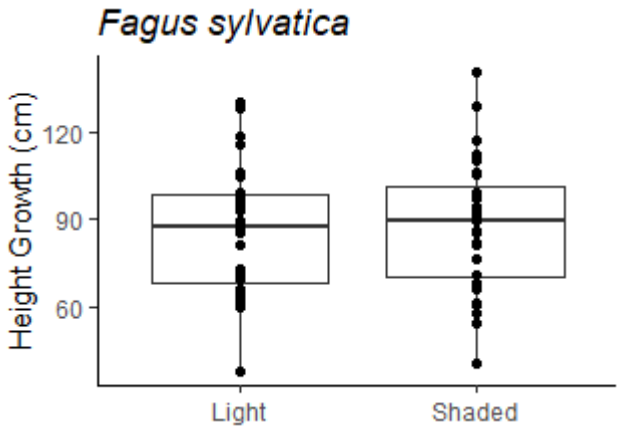


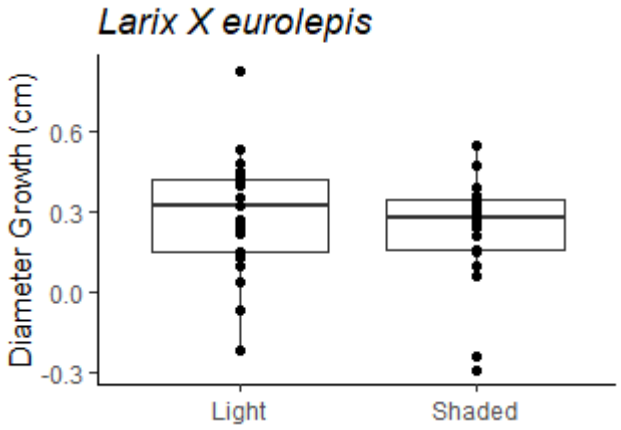


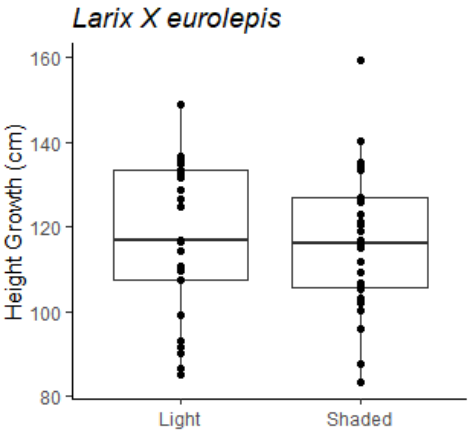


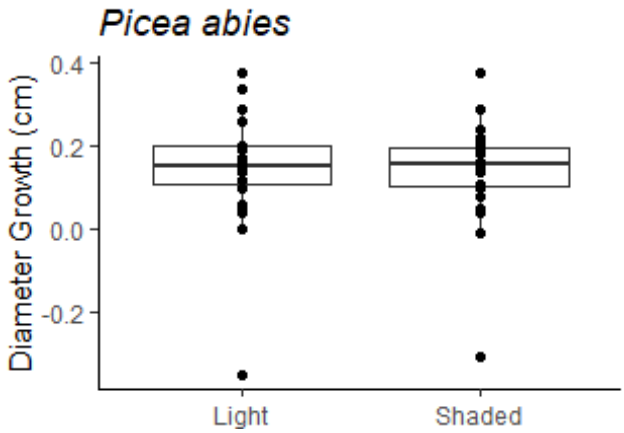


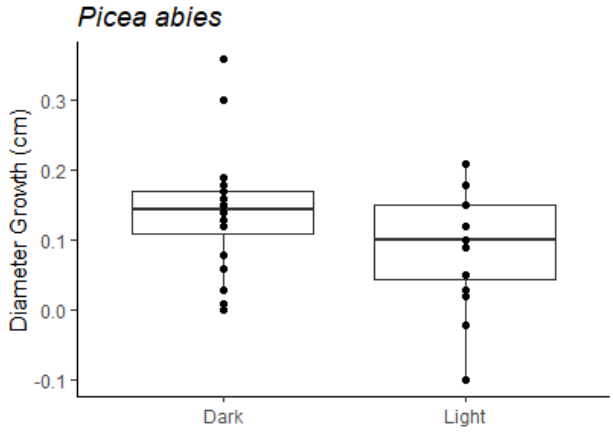

Fagus sylvatica
Diameter Growth (cm)
0.2
0.0
-0.2
Light
Shaded

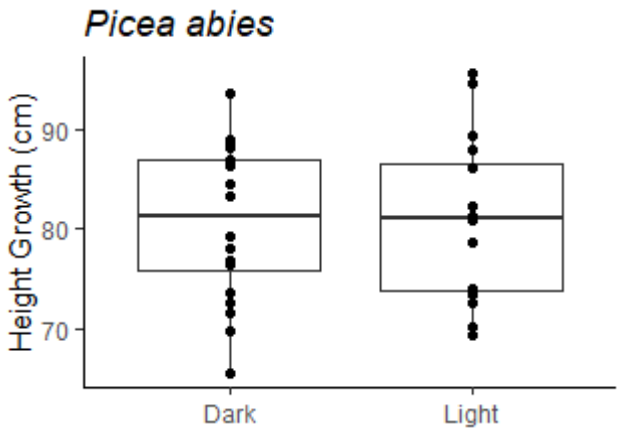

Fagus sylvatica
Height Growth (cm)
120
90
60
Light
Shaded

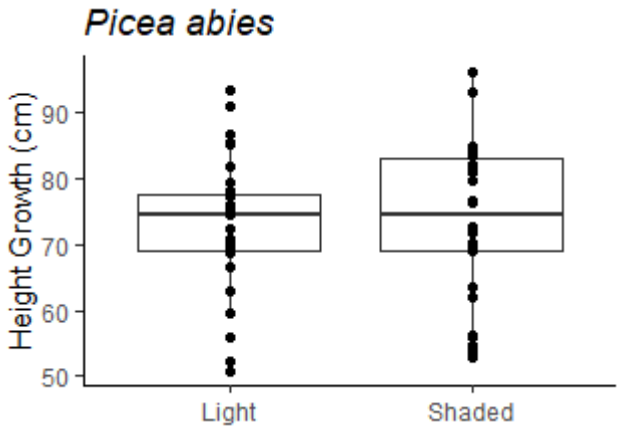

Larix X eurolepis
Diameter Growth (cm)
0.6
0.3
0.0
-0.3
Light
Shaded

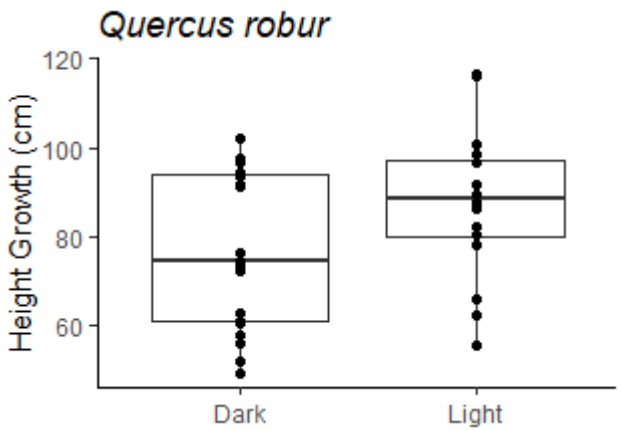

Larix X eurolepis
Height Growth (cm)
160
140
120
100
80
Light
Shaded

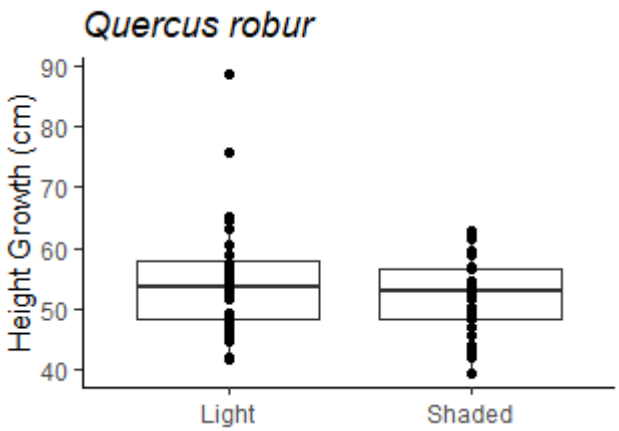

Picea abies
Diameter Growth (cm)
0.4
0.2
0.0
-0.2
Light
Shaded


Picea abies
Diameter Growth (cm)
0.3
0.2
0.1
0.0
-0.1
Dark
Light


Picea abies
Height Growth (cm)
90
80
70
Dark
Light


Picea abies
Height Growth (cm)
90
80
70
60
50
Light
Shaded


Quercus robur
Height Growth (cm)
120
100
80
60
Dark
Light


Quercus robur
Height Growth (cm)
90
80
70
60
50
40
Light
Shaded